PROCEEDINGS

OF

THE SYMPOSIUM

ON

INDIGENOUS DRUGS AND INSECTICIDES

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PREFATORY NOTE

The Council of the National Institute of Sciences of India, at their meeting, held in Calcutta, in January, 1952, decided to organize a Symposium on "Indigenous Drugs and Insecticides" to be held along with the Ordinary General Meeting of the Institute, at Bangalore in May, 1952 As response to the proposed Symposium was not satisfactory, it was postponed and in its place a lecture was delivered by the undersigned on "New Perspectives in Research in Indian Indigenous Drugs".

The Council revived the question of holding this Symposium at their meeting held in Delhi, in October, 1952, and the undersigned and Dr. H. S Pruthi were requested to organize it so that the Symposium may be held along with the Ordinary General Meeting of the Institute in Bombay, in August, 1953. Also at this meeting, a new Steering Committee, consisting of Dr. J. N Ray, Dr Mata Prasad, Dr. H S Pruthi, with the undersigned as the *Convener*, was appointed to organize it. The organizers made every efforts to secure the co-operation of experts on various subjects dealt with in the Symposium and it is a matter of deep satisfaction that a Symposium on "Indigenous Drugs and Insecticides" was held at the Royal Institute of Science, Bombay, on 6th and 7th August, 1953.

Dr. K. S. Krishnan, D.Sc , F.R S., President, National Institute of Sciences of India, opened the proceedings of the Symposium and then requested the *Convener* to conduct it as its Chairman. Many research workers from Bombay and other parts of India presented papers and took active part in the discussions. Several learned exponents of the indigenous system of medicine, including *Ayurveda* and *Unani*, were present Also many distinguished visitors from the academic and research institutions in the Bombay area participated in the meetings.

Over 41 papers were presented at the Symposium, but some of them are not included in this *Bulletin* as the authors had intimated their publication elsewhere. The papers published are classified under two major heads, viz. (I) Indigenous Drugs and (II) Insecticides. The first group is again sub-divided into six sections, such as, (A) Chemistry of Plant Products; (B) Pharmacology and Chemotherapy of Plant Products, (C) Biochemistry of Plant Products, (D) Therapeutics of Plant and Mineral Products, (E) Pharmaceutical Botany and Pharmacognosy of Medicinal Plants and (F) Papers of General Interest.

As Convener of this Symposium, I express my sincere thanks to all the authors for their interest and co-operation. I would also like to put on record my deep appreciation of the work of those including my colleagues of the Central Drug Research Institute, Lucknow, who helped me in making the Symposium a success in every way.

B. MUKERJI,

Convener,
Steering Committee.

Lucknow,
October 25, 1951.

ing personal hygiene to be observed during this time seems quite modern, even though these instructions were recorded near about the dawn of the Christian era. *Charaka* further prescribed calcium-rich diet during the menstrual period and also described the management of delivery and the use of forceps The operating room, according to him, should be clean and fumigated by disinfectant vapours before and after all operations. There is mention also of the use of a volatile anaesthetic. The technique described for operations of skin grafting, cataract couching, amputation, rhinoplastry, hernia, etc., are extremely instructive even to any student of modern surgery.

In *Charaka,* about 2000 vegetable remedies are described together with a few mineral drugs and animal remedies. The soil, the season and the gathering time of individual drugs of the vegetable kingdom are mentioned with such meticulous details that even modern students of [illegible] gain useful information from these descriptions. The methods [illegible] of drugs are fully described and bear a striking resemblance to those in use at the present time; even administration of medicaments by injection did not fail to attract attention. Natural and artificial feeding of the child, dental hygiene, methods of palpation of the radial artery, preventive medicine, etc., are to be found in various sections of the book.

Following *Charaka Samhita* and *Susruta Samhita,* a number of other valuable books on medicine and surgery were published. These are mostly of the nature of 'special' treatises, some dealing with anatomy and dissection of the human body in more detail (*Vagbhatta*) and others describing epidemics and their prevention (*Chakradatta*). It is not necessary to go into more details regarding the numerous evidences of the high standard of the ancient Hindu medical culture The examples cited will show that ancient Indian medicine was not based on pure empiricism, but was permeated with a scientific spirit, as evidenced by a desire, by observation and experiments and by induction and deduction, to probe into the secrets of nature and to build thereon a rational system of medicine.

Rise and Fall of Indian Medicine

From the time of Charaka and Susruta, to about 1200 A.D, Hindu medicine made good progress so much so that it not only became the acknowledged system of treatment all over India then known, but its influence extended to Egypt, Greece, Rome, Arabia and China. Available evidences indicate that [illegible] the Hindu physicians in the domain of drug therapy and [illegible] this period was far in advance to that of others Charaka's fame travelled into Arabia and at least part of his treatise on medicine appears to have been translated into Arabic. Avicenna, the renowned writer on Arabic medicine, quotes him as *Scirak* and Rahazes, who was prior to Avicenna, calls him *Scarak.* The early contacts of India and China are recorded in Buddhistic works There is also evidence to show that external trade existed between India and Rome for many centuries and this drug trade was in such enormous proportions that Pliny actually complained of the heavy drain of Roman gold to India for buying costly drugs and aromatic spices. There is reason to believe that many Greek philosophers like Paracelsus, Hippocrates and Pythagoras actually visited the East, and helped in the transmission of Hindu culture to their own countries. Jacolliot remarked "India, that immense and luminous centre in olden times, was in constant communication with all the people of Asia, and all the philosophers of antiquity went there to study the science of life."

After this period, however, the glories of Hindu medicine rapidly declined. During the invasion of India by the Greeks, Scythians and Mohammedans suc-

cessively, a good deal of the existing Ayurvedic literature was mutilated or lost Priesthood became the repository of all the medical knowledge and this probably was largely responsible for the introduction into the Ayurveda of many charms and amulets. Gradually, as a result perhaps of the Buddhistic doctrine of *Ahimsa,* touching of the dead body was considered sinful, and dissection as a basis for the study of anatomy and surgery was given up. With the advent of the Muslim conquerors, the Arabic system of treatment (Unani-Tibbi system) became the State system of relief and the Ayurvedic system was pushed to the background. During this transition period, there was a great deal of intermingling of the materia medica of the Ayurvedic system and the Unani-Tibbi system With the advent of the Europeans the decline of the older systems of medicine was marked with still further interchange of materia medica. The present indigenous medicine therefore is a hotchpotch produced by the irregular mixture of the ancient Ayurvedic medicine with Unani-Tibbi and Allopathic systems. Though hardly anything of the original system is existing to-day, the process of intermingling through centuries has left India with a rich heritage of a very varied materia medica, which is well worth careful investigation.

Ancient Medicine's Heritage to Modern Medicine

As an illustration of the extent of indebtedness of modern medicine, as practised in the second quarter of the 20th century, to ancient medicine of pre- and early Christian era, a drug map of the world is appended here showing the remote corners of the globe from where certain drugs were originally derived and which had since been incorporated in modern therapeutics in some form or other.

This will indicate in no uncertain terms that modern medicine still owes much to the people of the past for the accumulated knowledge of many remedies and cures While it cannot be denied that empiricism and superstition form a large part of the ancient indigenous systems of medicine, there are simultaneously many keen observations and experiences recorded which are of sterling value. An example can be found in the discovery of vaccination for the prevention of small-pox. Early records definitely indicate that Indians and possibly also the Chinese knew about the immunity from small-pox that could be attained from the inoculation of cow-pox debris In fact, Jenner, the discoverer of vaccination, obtained the information regarding immunity from the milk-maids and then conceived the idea of inoculation, which revolutionized medicine of the 18th century (1796). This does not, however, minimise the value of Jenner's discovery, but only shows that many apparently non-scientific doctrines and records of the indigenous system might possess a core of real truth, which can be analysed and confirmed by modern science. It has been repeatedly seen, particularly in the realm of ancient materia medica, that there are many century-old remedies which fully deserved the reputation accorded to them as 'cures', when judged by the critical yard-stick of modern pharmacology and therapeutics A very convincing example is the rediscovery of Ephedrine and Tubo-Curarine from the ancient Chinese materia medica of 5000 years ago and the African and South American folk-lore medicine of at least 2,000 years old respectively. Many such examples can be cited which will show the wisdom contained in the ancient systems that can still be gathered and absorbed into the modern progressive system of medicine. A quotation from Dr. Cummings, ex-President of the U S. Pharmacopoeia Commission is considered apt in this connection. "Any system of medicine or for that matter, any ancient usage or custom that has held its own for generations usually has something at the back of it, no matter how little it appears to be

supported by modern science. For generations the fact that the American Indian hunters always chose the liver and the whitemen the meat, when the animals they trapped or killed were divided, was quoted as proof of their ignorance and primitive development, yet in the last 5 years, the great nutritive value of liver has come to be recognized and is universally prescribed in cases of anæmia''

Problems of Research in Indigenous Systems of Medicine

One of the greatest difficulties of scientific research in the field of ancient medicine is that many learned exponents of these systems attach a great deal of sentiment to the teachings contained in the pages of the older treatises such as *Charaka* and *Susruta,* and consider these as 'inspired doctrines' incapable of improvement by modern researches and the 'man-made' science of the 20th century Any effort at critical evaluation, particularly by those not belonging to the Ayurvedic system of medical practice, is considered as nothing but therapeutic nihilism This School of thought favours the wholesale revival of the old systems with all their paraphernalia of 'compounded medicines', 'crude methods of pharmacy', 'polyglot recipes', etc Modern practitioners of the Western system of medicine, on the other hand, have frequently pooh-poohed the idea of research in the indigenous systems, which are considered to be nothing more than an assemblage of facts and information based largely on folklore, and which does not deserve the serious consideration of modern science What is needed today in any discussion about ancient systems of medicine is not sentimental or extremist thinking but a balanced, critical and open-minded attitude which would permit a reasoned appraisal of the teaching and observations of the indigenous systems and adoption into modern medicine of those legacies only which can be fully supported by modern science. A stagnant and static system for well nigh 1,500 years is bound to get mixed up with many things of questionable value and doubtful utility. There is no harm in recognizing this and devising ways and means of revitalizing indigenous systems of medicine, taking full advantage of the modern developments in physico-chemical and biological sciences Only by such means can any progress be achieved.

Planning of Research in the Indigenous Materia Medica

The question now arises as to what is the best way of investigating the rich materia medica left to us as the heritage of the indigenous systems of medicine. From the empirical knowledge of a crude drug (stated to be a good remedy) to its use in rational scientific medicine, is a long way and must pass through (1) Botanical indentification, (2) Chemical examination, (3) Pharmacological and toxicological assay and (4) Clinical trials. All stages through which modern scientific investigation should proceed will be discussed more fully in a later chapter of this review. It is important to recognise here that the work involved is of great magnitude and complexity.

In India, study of the indigenous materia medica according to modern scientific lines (as distinguished from purely 'clinical trials' carried out in the late 19th and 20th century) was first started at the School of Tropical Medicine (Chopra and [illegible]), Calcutta, and at the Haffkine Institute, Bombay (Caius, [illegible]) in the second decade of this Century. The problem was approached from the following angles:—(1) Investigation of the possibilities of utilization of pharmacopoeial and allied drugs growing in India in place of the 'official' ones mentioned in the British Pharmacopoiea and other recognized pharmacopoeias. This led to the finding of several allied species of plants of known value, such as, the various species of Hydnocarpus, Indian Senega,

Indian Digitalis, Belladonna, Squill, Gentian, Rhubarb, etc., as substitutes for the corresponding pharmacopoeial species; (2) The trial of specifics for various diseases, such as Holarrhena, Rauwolfia, Butea, Alstonia, Caesalpinia, Adhatoda, Punarnava, Melia, etc., for dropsy, dysentery, malaria, etc Some of these have received widespread attention and are adopted in modern medicine while others have been discarded as being of little value; (3) The research for new active principles, especially drugs of alkaloidal character, glucosides, tannins, etc, such as Ephedrine, Ajmaline, Berberine, etc. and (4) New sources of therapeutic agents of proven value, such as the various solanaceous plants used in the preparation of atropine, and new sources of Santonin, Ephedrine, etc.

Pharmacopoeial and Allied Drugs

During the last 30 years which has elapsed from the time R N. Chopra initiated this work, much ground has been covered particularly with regard to making India self-supporting so far as pharmacopoeial and allied drugs are concerned. A large number of drugs which grow in this country are known both to Indian and Western medicine, and their preparations and actions in many cases are also known. The research work that has been carried out in this field can be diverted into two main channels: Firstly, there are many drugs of established therapeutic value which are in use in the pharmacopoeias of different countries. The majority of these, e.g, Aloes, Artemisia, Ephedra, etc., grow wild and in great abundance in many parts of India, and a certain number such as Rhubarb, Scilla, etc., are even cultivated Some of these are collected and exported to foreign countries and come back in the form of standardized pharmaceuticals and active principles in pure condition, probably at a price hundred-fold higher than that of the crude product. A host of other plants grow, mature and eventually die without being put to any effective use whatsoever There are numerous examples which have been dealt with by several workers including Chopra, in books and research papers on this. More than 50 items can be easily recorded under this category. Besides these, there are a number of pharmacopoeial drugs which are widely used by the medical profession, but which do not naturally grow in this country. They thrive, however, when they are cultivated under proper conditions in suitable parts of the country Digitalis, ipecacuanha, eucalyptus, cinchona, jalap, mentha, etc, may be cited They were introduced into India many years ago and are doing well.

Secondly, a large number of plants grow in India which, though not exactly the same, have properties and actions similar to the imported and often expensive remedies and would form excellent substitutes. Not infrequently there are some closely allied species which are pharmacologically just as active, e.g, *Colchicum luteum* for *C. autumnale, Urginia indica* for *U. maritima, Picrasma quassioides* for *P. excelsa,* etc The properties of many of these plants have been worked out on scientific lines and they are now being brought into use. A complete list of these substitute drugs has been published recently by the (12)Council of Scientific and Industrial Research. Detailed description, habitat, availability, economic and commercial aspects of the more important members in this group have been incorporated in the 3 volumes of "Wealth of India"(13) also published by Council of Scientific and Industrial Research.

Considerable amount of work has been done by distinguished chemists and pharmacologists in the various university laboratories, particularly in the departments of chemistry and pharmaceutics, the School of Tropical Medicine, the Haffkine Institute the now defunct Bio-chemical Standardization Laboratory, Drug Research Laboratory, Kashmir, etc., with regard to the active

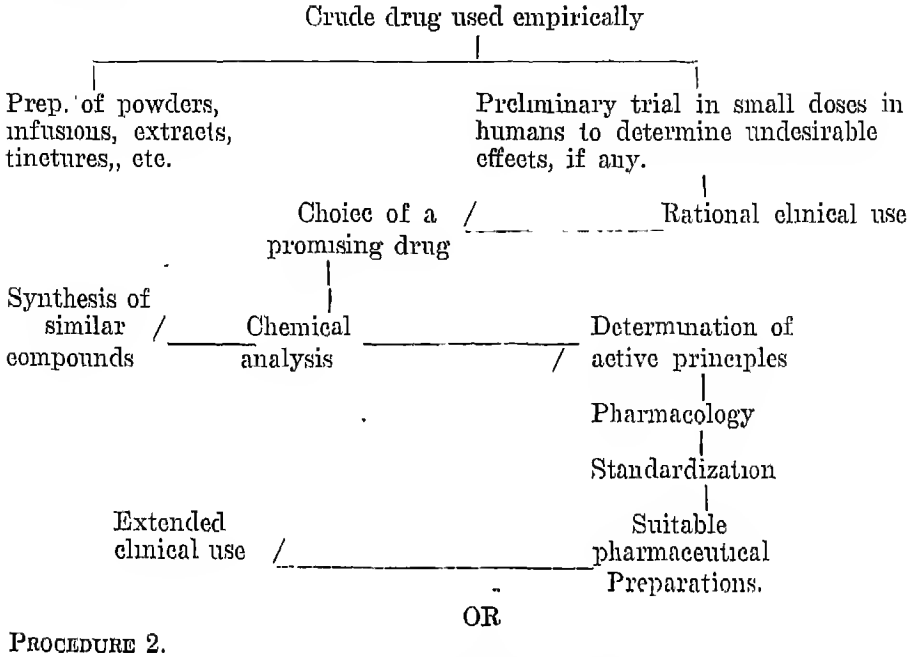
ingredients of these drugs, determination of the percentage composition, purity tests, assay proeedures, and establishing their pharmacological action. Pharmaceutieal preparations and active alkaloids, etc., from source materials are now manufactured from these indigenous products to the immense advantage of the country. The data with regard to about 180 items have come to such a scientific level that it has been possible for the Government of India in the Ministry of Health to publish, in 1946, the first 'Indian Pharmacopocial List'(14) which is a book of standards for Indian drugs of the same status as the British Pharmacopoeia. From this nucleus, efforts are also being made through a representative committee under the Health Ministry to compile the first 'Indian Pharmacopoeia', a venture when successfully completed would place India in the pharmaceutical and scientific map of the world. All civilized countries worth the name have their national pharmacopoeias, and new independent India cannot do without it. Recently the writer has published, under the auspices of of the Council of Scientific and Industrial Research, the first Indian Pharmaceutieal Codex(15) which provides 193 monographs and similar number of 'Formulary' giving pharmacognostic, chemical, pharmaeological and therapeutic data on indigenous drugs with their preparations and dosage, for the use of medical and pharmaeutieal professions. The scope of these and similar publications will naturally be considerably extended as time goes on and as more and more authoritative data on indigenous drugs become available from various active researeh centres in India. The time does not appear far off when it would be possible to bring into use for the treatment of minor maladies many of these standardized indigenous drugs which will provide equally good remedies for symptomatic treatment and the need for import of galenicals, tablets, simple proprietary preparations, etc., would be completely done away with. Mueh ground has already been covered by Indian manufacturers of drugs in this direction.

Drugs Used in the Indigenous Medicine

The second proposition of popularizing and introducing new drugs to western medicine is a more difficult one. It is believed that out of a very large number of drugs used in the indigenous system of medicine for centuries past, and still in use, there must be some at least which might deserve the reputation they have earned as 'eures'. Since the period of decay and recompilation of old Indian systems of medicine, many of the effective remedies have been lost and a number of others have come into use. The result is that in the indigenous system at the present time, almost every plant and shrub growing in the country has ascribed to it some medicinal virtue. These beliefs in some cases originate from teachings of ancient commentators and are based on preliminary elinieal data, but others have no foundation whatsoever. In this way remedies have multiplied without proof but by belief, and as they hail from all parts of India, no one seems to have eorreet notion about their uses and properties. The employment of a large number of them would thus appear to have been based on empirieal ovidence handed down from generation to generation. A thorough and complete research into all these drugs would constitute the lifework of innumerable chemists, pharmacologists and clinicians. From the empirieal knowledge of a crude drug to its use in the rational scientific medicine, is a long way and must pass through (1) botanical identification, (2) chemical examination, (3) pharmacological and toxicological assay and (4) chemotherapeutie and clinical trials. It is sometimes convenient and less time-consuming to start from clinical trials first and then proceed to further laboratory study and analysis. A diagrammatic representation of the steps involved is given below:

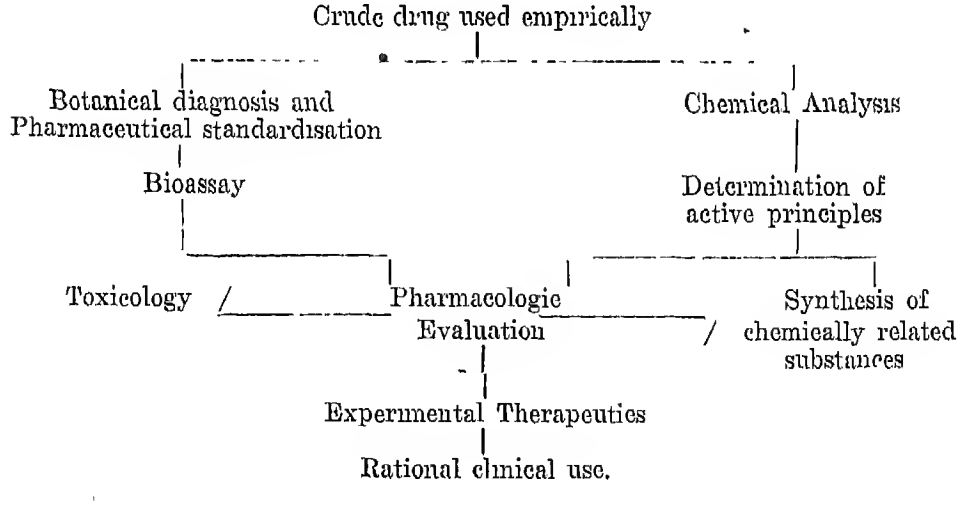
SCHEMATIC PROGRAMMES NECESSARY IN THE EVALUATION OF A DRUG

PROCEDURE 1.



OR

PROCEDURE 2.



In whatever manner the investigative approach on indigenous drugs is made, successful or even satisfactory work is time-consuming and requires a 'team-work' of several groups of scientists, each expert in his own fields of specialisation but integrated and co-ordinated for solution of one or other problem at one time. No haphazard methods of approach by individuals or even by single institutions with inadequate resources are likely to succeed.

As properly trained personnel and facilities needed for this type of work did not exist in India in the thirties except in the School of Tropical Medicine,

Calcutta, and partly in the Haffkine Institute, Bombay, most of the authentic data have naturally emanated from those centres. With the growth of pharmaceutical institutions and many chemical laboratories in universities and also in pharmaceutical concerns, some significant progress has been recorded during the last decade or so. Interestingly enough, several renowned research centres in Great Britain, Switzerland and America have also taken up intensive study of Indian indigenous drugs. Such work has brought into prominence the merits and qualities of certain drugs such as *Holarrhena anti-dysenterica, Rauwolfia serpentina, Butea frondosa, Alstonia scholaris, Caesalpinia bonducella, Adhatoda vasica, Bacopa herba, Daemia extensa, Cissampelos pareira, Terminalia arjuna, Psoralea corylifolia, Sida cordifolia, Swertia chirata, Andrographis paniculata, Plantago ovata, Thevetia neriifolia, Rivea cuneata,* etc, etc. *Holarrhena* (Kurchi) has come to stay as a reliable anti-dysentric remedy, particularly in subacute and chronic forms of amoebiasis complicated with what has often been described clinically as 'post-dysenteric abdomen'. Standardized liquid extract and a preparation, Kurchi Bismuth Iodide, have been accepted as recognized remedies in the Indian Pharmacopoeial list and Indian Pharmaceutical Codex. During the Second World War, the latter preparation was largely used in the theatres of war in the near and Far East with satisfactory results. *Rauwolfia,* an old drug used in the empiric medicine of India for centuries as a purgative, anthelmintic and antidote for snake and insect bites and more recently in clinical medicine as a hypotensive agent and as a sedative in the treatment of insomnia and certain forms of insanity, has recently been introduced into clinical medicine in America as one of the best remedies for the treatment of hypertension. The first pharmacological paper on this sovereign remedy was published as far back as 1933 by Chopra, Mukerji and Gupta(16). Due to lack of interest and proper pharmaceutical exploitation methods then available in India, no advantage of the findings could be taken. In 1950 and 1951, the writer had the opportunity of lecturing on the subject in America and largely due to this and many publications on the subject from India, renewed interest was aroused resulting in its acceptance as a recognized therapeutic item. Recently the discovery of 'Reserpin', the hypnotic principle of *R. serpentina,* has created a sensation in the field of researches on *Rauwolfia* and quite a number of workers all over the world are engaged in working with different species of *Rauwolfia,* such as *R. serpentina, R. canescens, R. vomitoria, R. obscura, R. caffra, R. semperflorens, R. densiflora,* etc. and isolated not less than 40 alkaloids of varying degrees of physiological importance. Recent work on *C. pareira* at the Central Drug Research Institute, Lucknow, has given indication that from this Indian plant, available in plenty in the lower ranges of the Himalayas, a substance can be found which is as good a smooth muscle relaxant as d-tubocurarine chloride From *T. neriifolia,* a pure glycoside has been obtained having properties and uses similar to Digitalis. In *A. paniculata* and *S. chirata,* we have bitters and cholagogues which can defy the best items of their class in foreign pharmacopoeias. The use of *P. ovata,* introduced by Unani medicine to India, has now been accepted all over the world.

Unfortunately, out of approximately 100 items so far studied scientifically, a few showed a certain amount of activity, but they were not found to be superior to the drugs already possessed by the foreign pharmacopoeias. The majority of drugs investigated have been found to be of questionable value and of doubtful utility, and, therefore, had to be discarded. There is still a vast field (over 2000 remedies) to be covered and there is every likelihood that the proportion of worthless remedies would be much more than acceptable ones.

Even if a few successful finds are made, it is well worth probing deep into this mine of knowledge.

Identification of Indigenous Drugs

Closely associated with any research effort on Indian indigenous drugs is the problem of their correct identification Many of the remedies mentioned in the old books baffle recognition and identification, and one cannot be certain from the description whether the specimens obtained are of the particular drug described No amount of verbal description of these drugs as given in the books will enable the botanists to identify some plants and parts which even in themselves do not invariably present the same characteristics. The results is that there has been a good deal of confusion, many drugs are being sold under various names, different drugs under the same name and even experienced workers cannot say with certainty which is the authentic specimen meant in the old texts The inevitable result has been that much of the earlier work in this field have suffered from inaccuracy and mis-statements.

The problem of identification became particularly important at the time of compilation of the first Indian Pharmacopoeial List in 1944-46 As one of the principal workers in the Committee entrusted with the work by the Health Ministry, the writer organized a systematic pharmacognostic study of Indian drugs to help correct identification of medicinal plant parts and drugs in powder form This work resulted in the production of two Bulletins[17] which have been published by the Health Ministry, Government of India. With the establishment of pharmacy colleges and pharmaceutics departments in various universities, researches on plant pharmacognosy are proceeding apace, thus opening up a new line of investigation in India Several Indian Medicinal plants are also being studied histologically by foreign workers, in Great Britain, America and Switzerland, such as, Indian podophyllum, Isphagula, Indian Squill, Indian Rhubarb, Indian Belladonna, Rauwolfia, etc.

Cultivation of Medicinal Plants and Production of Crude Drugs

Growing interest in medicinal plants both in India and abroad and indiscriminate exploitation of such products as Rauwolfia, Belladonna, etc., have during the last decade or so resulted in an almost complete depletion of many of our valuable natural resources both in the field of medicinal plants and condiments and spices. A great set-back has also come due to seceding of certain territories rich in medicinal plants to Pakistan. It is also well known that the collection of medicinal plants from nature does not give a uniformity of product. There is either deliberate or innocent adulteration because of lack of proper knowledge on the part of collectors and collection is often done without taking into consideration the proper season and other factors. R. N. Chopra has again given a lead in this direction by starting an Experimental Drug Farm in Srinagar where researches are being conducted for the cultivation of *Pyrethrum, Atropa belladonna, Atropa acuminata, Digitalis purpurea, Digitalis lanata* and *Podophyllum emodi.* The Indian Council of Agricultural Research, through its 'Medicinal Plants Committee', are also sponsoring such activity in several zones in north-eastern, northern and southern India. It is encouraging to see that attention is being paid not only to the cultivation of indigenous plants but also towards the introduction of exotic plants such as *Datura innoxia* from Mexico and *Duboisa species* from Australia as better sources of hyoseyamine, *Urginea scilla* from Mediterranean coasts and *Coriandrum sativum* and *Foeniculum vulgaris* from Russia and Germany, and *Heliopsis longipes* from Mexico as a source of an active principle called

'scabrin' which is 2½ times as toxic as pyrethrin to the house flies. If such efforts are made by a devoted band of research workers aided by agricultural and horticultural experts, the potential wealth of the country would increase many-folds and our dependence for these on other lands would be a thing of the past.

Improvements of economic plants is a fascinating study and is worth some mention here as it is directly concerned with the production of better crude drugs in India. Grafting, selection, hybridisation, induction of mutagenic variants by radiation and chemical agents, and production of polyploidy by colchicine treatment are some of the methods employed in this direction. Much work has been done in other countries but in India only a beginning has been made towards bringing about improvements in crop plants and few medicinal plants such as *Hyoscyamus niger, Datura metel, Ocimum basilicum,* etc. This promising field of investigation is yet largely unexplored It is time now that the State should exercise control over the export of crude drugs of established therapeutic properties. This will enable India to have a good market for different preparations from these drugs in foreign countries

Re-investigation of Well-known Indigenous Drugs

Recent studies on medicinal plants in Europe and America have tended to indicate that there is room yet for looking 'backward' on some of the items of old-world materia medica. Though known for several centuries, apparently the last word on the properties and uses of such drugs as *Podophyllum, Glycyrrhiza, Veratrum* etc., has not been said. A very significant example of the rich dividend which reinvestigations of some of the older crude drugs has yielded is provided by *Podophyllum* (the rhizomes and roots of *Podophyllum peltatum* L.) *Podophyllum* and its resin have long been known to cause irritation of the mucous membranes and even of the skin of workers handling them, and it may have been knowledge of this fact which initiated the attempts to destroy soft warts (Condylomata) by means of the resin The first published account of this treatment was given by Kaplan[18] in 1942 and later (1945)[19] strikingly successful results were reported by topical application of a 25 per cent suspension of podophyllum resin in mineral oil in venereal warts. This treatment is now well established and justifies the inclusion of Podophyllum resin in the International Pharmacopoeia[20]. Though this work has been done on American Podophyllum, it is possible that resins from Indian Podophyllum (*P. hexandrum*) will contain the same constituents (podophyllotoxin, α & β peltatins) perhaps in greater quantity Recent analysis of Indian Podophyllum[21] has reported the presence of podophyllotoxin but does not mention the peltatins; however, these may be discovered on further search.

Dutch workers[22, 23] have recently reported the beneficial effects of extract of liquorice for gastric ulcers, they also noted that about 20 per cent of the patients developed cardiac asthma during treatment. Further investigation showed that the extracts have an action *by mouth* similar to that of injections of deoxycortone, causing sodium retention and potassium loss and they report beneficial effects of the treatment in Addison's disease. One of the components of liquorice is glycerhetic acid which is a polyterpene whose structural formula shows a striking resemblance to the Cyclopentanophenanthrene steroids.

Another interesting development in the recent study of Digitalis is the emphasis on its 'cardiotonic' rather than on its 'cardiotoxic' properties and the reported discovery of a new glycoside 'digicorin'[24, 25]. This glycoside, which has low toxicity, is claimed to possess the curative action of digitalis as

distinct from that of the better known glycosides which are largely cardiotoxic. It can be extracted from the leaves of *Digitalis purpurea* and *D. lanata*.

Recent work[26, 27] on the anthraquinone group of purgative drugs has drawn attention to the importance of the form in which the anthraquinones occur in the crude drug Satisfactory chemical and biological methods of assay which have been developed show that the anthracene derivatives are highly active as 'anthranol' glycosides, less active as free anthranols and much less active as free anthraquinones Studies on these lines made on Senna, Rhubarb, Cascara and Aloes tend to clarify the exact nature of the active ingredients in these drugs. This will now enable preparations of potent and stable galenicals for therapy which were not possible before.

Recent work on the American veratrum, *Veratrum viride* has shown its usefulness for the treatment of hypertension and it is very probable that the European veratrum or white Hellebore has a similar action[28]. The pharmacological and clinical evaluation of veratrum has been greatly hindered by the complex chemistry of the drug. Already 15 alkaloids have been reported and some work on these have been done. Further studies might throw more light on its nature of action.

It is difficult to form an unprejudiced opinion of the favourable reports on the deodorising properties of chlorophyll derivatives because of commercial claims calling attention to this new miracle material from Nature's own store room However, there is a remarkable unanimity in the conclusions reached by reliable research workers on the deodorising value of the chlorophyll derivatives and also on their healing effects in the treatment of war wounds.

Indigenous Drugs of Non-Indian Origin

Since the discovery of ephedrine from the Chinese drug, *Ma huang*, the Chinese materia medica have attracted attention of many western research centres, as also by Chinese workers in the same way that indigenous drug research is being conducted in this country. Nearly 20 years ago the author found two flourishing schools of study in Peiping and Shanghai engaged in the scientific appraisal of the claims of numerous Chinese indigenous drugs. No outstanding drugs which can be recommended for inclusion in the International Pharmacopoeia has thus far been reported but according to Tonkin & Work[29] a drug known as 'Chang-shan' has been proved to be an anti-malarial more or less of the same potency as quinine. During the Second World War when China was almost completely cut off from the allies, this drug was reported to have been used with considerable success.

A decoction of dried fruits of *Ammi visnaga* has been used for centuries in the Middle East as a diuretic and an anti-spasmodic for ureteral stone and was included in the Egyptian Pharmacopoeia (1934). Later work indicated that the most active constituent of the drug was 'Khellin' and [illegible] pharmacological investigations have confirmed its effect on the coronary [illegible] Clinical trials have shown that khellin is particularly useful in angina and also in whooping cough.

The fruits of the closely related plant, *Ammi majus* have long been used by the Egyptians for the treatment of leucoderma. Research work has confirmed that the condition can be cured by the oral administration of the drug and subsequent exposure to sunlight of the white patches of skin. A crystalline active principle, ammoidin has also been isolated.

Rutin, now a well-known glycoside, originally derived from *Sophora japonica lens*, has since been reported from 40 different species of plants including wheat, tobacco, elder and forsythia Until 1942, it was a laboratory

but now it is coming to be increasingly employed in the treatment of capillary fragility Recently, an accidental discovery by a group of pharmacologists(30) had led to what may be an important use of rutin in the treatment of the after effects of exposure to atomic radiation.

Fundamental Research on Plant Products

Considerations of space necessitate only a brief reference to work on the place of active principles in the biology of the plant producing them. Moreover, it is only recently that attention has been paid to this aspect of the study of medicinal plants so that little is yet known on the subject. The alkaloids have been more studied in this connection than almost any other group. James(31) has recently reviewed our present knowledge and concludes that at least for some plants the alkaloids are formed from the 'soluble nitrogen' pool, which normally consists of amino-acids and amines. These intermediate compounds are removed from the pool to build up proteins and in like manner, the breakdown of proteins results in the return of them to the pool. In alkaloid-producing plants this two-way traffic is partly diverted to alkaloid production and break-down. Such a theory explains why alkaloids are frequently found in actively growing tissues where protein metabolism is active, and why fertilizers which increase the growth of plant also produce a corresponding increase in the alkaloidal content.

Very similar type of fundamental work is also being carried out with glycosides and also with many other plant vitamins. The role of the cyanide group, which occurs in several drugs containing cyanogenetic glycosides, has also received attention lately because of its possible effect on enzyme systems or in nitrogen metabolism.

Newer Orientation in Research on Indigenous Drugs

So far, Indian drug research has largely been concentrated on the research for active principles in the vegetable materia medica. The animal drugs and the various remedies for deficiency diseases and inorganic mineral elements mentioned in the indigenous systems have not received equally careful attention While modern medicine is turning to liver, stomach, insulin from the pancreas, fibrinogen from the lung and blood, albumin and gammaglobulin from blood vitamin A from the eye and fish liver oils, adrenaline, thyroxine, parathormone, plasma, serum, vaccines, choline, etc, it is remarkable to find that many animal tissues, and organic glands such as, blood, bones, neck glands, heart, liver, lung, marrow, kidneys, pancreas, bile, urine, etc., had been freely used in the indigenous systems of medicine. Mention has also been made in ancient materia medica of crude remedies such as *mung* beans, walnut, pigs liver, etc., for nightblindness and a condition akin to 'beri-beri', which would tend to indicate that the ancients had made keen observations on conditions produced by vitamin deficiencies Similarly, the recommendation for the use of a large number of green and other plant sources containing vitamin C, such as capsicums, brassicas, pumelo and mustard leaves, in the diet of certain types of dental affections and skin conditions cannot be brushed aside as simply fortuitous coincidence. Indigenous remedies claiming to have power to prevent sterility and increase human fertility on the one hand and acting as oral contraceptives on the other are often associated with magical ideas but in view of the increasing volume of recent scientific work in this field(32, 33) it is hoped that information may be forthcoming whereby these claims can at least be partly substantiated. Present knowledge justifies to some extent the claims for human placenta, marrow of animals, pig's pancreas and testicles, and pregnancy urine

as aphrodisiacs and sex stimulants. The importance of inorganic mineral elements in foods and their function in maintaining body metabolism was apparently recognized and would be seen from the fact that many recipes are described containing 'bone powder', 'bone marrow', etc, which are rich in calcium salts. Many foods rich in copper, and iron in organic form were recommended in diseases which appear to be conditions caused by anaemia or pregnancy. Deficiency of iodine was definitely known to cause goiter and this has been used repeatedly in the treatment of 'swelled neck'. Further, it has often been claimed that the action of a drug in a fresh condition is different from the action of a drug in a dried and stored condition. If this view is correct, most of the findings recorded by modern methods of chemical, pharmacological and clinical trials of indigenous drugs would need re-examination. While it is very difficult to find any palpable difference between the action of a fresh drug and a stored crude drug, excepting perhaps that the fresh drugs would contain certain amount of vitamin C, particularly if green parts of plants are used, this in itself is not adequate to explain the various changes that have often been reported by the savants of Ayurvedic system of medicine after the administration of fresh juices. If there are any changes at all, which it is difficult to prove by the application of modern science of physics and chemistry, it is nevertheless a very age-old and interesting observation which needs to be critically surveyed It is known for example that the reaction of a total crude drug may be quite often different from its isolated active principles; thus, the action of opium is definitely different from the action of morphine and similarly, the action of a large number of crude purgative drugs is different from the action of its isolated active principles, e.g., the action of 'aloin' and 'sennosides A & B' as distinguished from the *whole drug*, Aloes & Senna All these call for a new study of the old empirical methods from the angle of modern organotherapy, deficiency diseases and imbalance of metabolism which has not so far been done Such evaluation by new standard of modern physiology and biochemistry of both foods and drugs (as no distinction is made between foods and drugs in ancient medicine) is likely to lead to further interesting results than have so far been obtained through a study of only the active chemical principles of drugs and their pharmacotherapeutic application. Maintenance of a healthy balance of the normal body processes is just as much a function of medicine (and this aspect was more important in ancient medicine) as drastic curative treatments through the introduction of potent foreign substances into the system

Conclusion

This review indicates that research in the vegetable materia medica is yielding and may yet yield much information useful to medicine. This is true not only in India but also in several other areas of the world where competent scientific talent has been harnessed towards this type of work. India's vegetable materia medica offers a vast field of study and all that has so far been done can be considered to have touched only the fringes of a complex problem—a problem which is probably of considerable interest to all countries bordering on the Indian Ocean, as close affinities in the occurrence and distribution of similar medicinal plants are evident throughout this part of the world.

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GROUP I.

INDIGENOUS DRUGS.

Section A.

CHEMISTRY OF PLANT PRODUCTS.

STEROID HORMONES FROM INDIAN DIOSCOREA PLANTS

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(Communicated by B. Mukerji, F.N.I.)

Introduction

In 1903 when Windaus started his work on the sterols or even in 1912 when Wieland started his work on the closely related bile acids few appreciated the usefulness of such kind of work, but as it is to-day the steroid chemistry is a distinct and extremely important branch of organic chemistry. The accumulated literature in this branch may be described as enormous and the subject has become extremely complicated due to the presence of various types of stereoisomerism, a clear conception of stereochemistry being essential for workers in this field. The steroids are organic compounds occurring in nature having a reduced cyclopentenophenanthrene ring system (I). They may be classified as follows:—(1) sterols, e.g. cholesterol (II); (2) bile acids, e.g. cholic acid (III); (3) steroid hormones including both (a) sex hormones, e.g. progesterone (IV) and (b) cortical hormones, e.g. cortisone (V); (4) aglycones of heart poisons, e.g strophanthidin (VI); (5) toad poisons, e.g. bufotalin (VII); (6) aglycones of steroid saponins (steroid sapogenins), e.g. diosgenin (VIII); (7) steroid alkaloids, e.g solanidine (IX). Amongst these different classes of steroids the hormones appear to be the most useful as these are, now a days, in great demand for therapeutic purposes. Preparations of the various useful steroid hormones on a large scale are beset with great difficulties. Usually there are three ways of preparation of an organic compound:—(i) by total synthesis, (ii) by direct isolation from some natural source and (iii) by partial synthesis from a closely related naturally occurring organic compound which is available in sufficient quantities.

Although in recent years various papers have appeared on the total synthesis of steroid bodies large scale preparation of steroid hormones by total synthesis has not yet been materialised chiefly due to the formation of various unwanted isomers at different stages and the difficulties involved in their separation. Isolation of these hormones may be effected from the glands of animals as also from urine but such preparation invariably involves extremely elaborate procedure as these are present in glands or in urine in minute amounts only, e.g. 1,000 lbs. of beef adrenal glands yield about 500 mg. of cortisone. The third method, that of conversion of any of the easily available steroids into the desired hormone appears to be the best from the view point of large scale preparation. The more easily available steroid bodies are the

sterols, the bile acids and the steroid sapogenins and a large volume of work has already been carried out on the conversion of these into the various steroid hormones. Amongst the steroid sapogenins diosgenin is now a days largely utilised for the purpose. It was first isolated from the yams of *Dioscorea tokoro* Makino of Japan by Fujii and Matsukawa in 1936. Later, Marker and his collaborators carried out an extensive work on the sapogenins of Mexican *Dioscorea* plants and isolated various sapogenins including diosgenin. Diosgenin was also isolated by them from a large number of American *Trillium* plants as also from *Balanites aegyptica* We are carrying out an extensive investigation of the Indian *Dioscorea* plants under the Indian Council of Medical Research, with a view to find out a rich Indian source of diosgenin or any other steroid sapogenin which may be useful in this respect.

The Indian Dioscorea Plants

The *Dioscorea* plants are climbers which have characteristic tubers known as yams. The plants have often prickly stems, the leaves are reticulate and petiole often angled. Flowers are small, unisexual, dioecious or monoecious in separate spikes. The yams vary from species to species in size and shape. The flesh of the tubers are often soft but there are some having hard woody tubers. Some of the yams are edible and are used as cheap substitutes for potatoes. The yams of a number of plants are inedible and poisonous. The poisonous characters are in most cases due to the presence of saponins and in some cases due to the presence of toxic alkaloids. Inedible tubers are often taken by Sonthals, Kols, Bhils and Paharias after removal of the poisonous principles by thorough washing of the sliced or macerated tubers with hot water. The product is usually used as a substitute for flour. Notable poisonous yams amongst the Indian Dioscorea plants are those of *D. hispida, D. deltoidea,* and *D. prazeri. D. hispida* is known as *marapashpoli* or deadly strangle cake in Western India on account of its poisonous character. The Bhils poison tigers by placing pounded tubers in the carcases of 'kills'. *D. prazeri* is called *kencheong* by the Lepchas and Paharias call it *kukur torul* or dog's yam. Indian names of *D. deltoidea* are *kns, kildri, kithi, krithi, krits, krish* etc. Both *D. prazeri* and *D. deltoidea* are used as soap specially for washing wool and silk, also for washing hair to remove lice and as fish poisons. Some of the Dioscorea yams are used in the indigenous system of medicine as diuretic. The plants of this family have been subdivided into two subgroups depending on whether the plant twines to the right or to the left.

At least thirty different species of this plant grow in different parts of this country, some in the hills, others in the plains but mostly in jungles. Some of the edible varieties are even cultivated and yams of these are sold in local markets. Till now it has been possible to collect yams of nineteen different species of these plants mostly through the various forest offices. These are—*D. alata, D. glabra, D. oppositifolia, D. Wallichii, D. bellophylla, D. pubera, D. nummularia, D. aculeata, D. esculenta, D. hispida (Indian), D. pentaphylla, D. tomentosa, D. prazeri, D. bulbifera (Indian), D. deltoidea* and *D. sativa.* Of these the first eight belong to the right twining subgroup and the rest to the left twining subgroup. Three of the yams could not be identified properly.

General Procedure and the Results Obtained

The yams after collection were allowed to sprout by keeping in a humid atmosphere. The plants with the yams were then sent to the Indian Botanic

(I)
(II)
(III)
(IV)
(V)
(VI)
(VII)
(VIII)
(IX)
(?)

Garden for identification. The collected yams were cut into small pieces and dried in air. Loss in weight during drying which represents the moisture content of the particular yam, was noted. These are stated in table I.

TABLE I

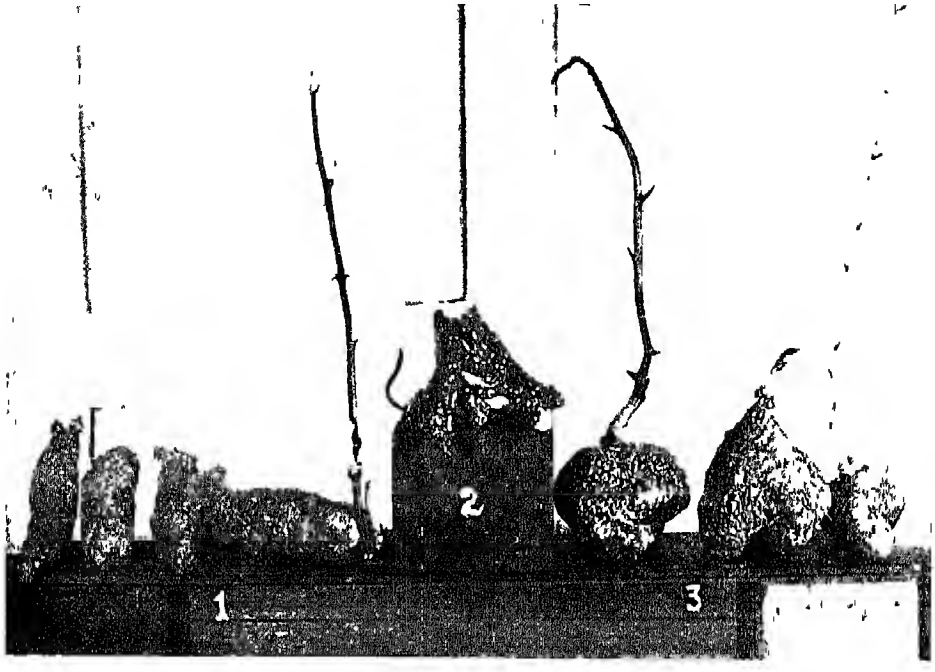
Plants Twining to the Right	Moisture Content. %	Saponin Content.	Plants Twining to the Left.	Moisture Content. %	Saponin Content.
D. alata	82	low	D. esculenta	57.5	fair
D. glabra	66.5	traces	D. hispida (Indian)	55	traces
D. oppositifolia	55	nil	D. pentaphylla	70	nil
D. Wallichii	64	traces	D. tomentosa	72	traces
D. bellophylla	—	traces	D. prazeri	35	very high
D. pubera	79	nil	D. bulbifera (Indian)	68	low
D. nummularia	65	traces	D. deltoidea	20	very high
D. aculeata	69	nil	D. sativa	—	traces
Unidentified Species of Dioscorea.					
No. 1	78	nil	No. 2	60	nil
No. 3	59	low			

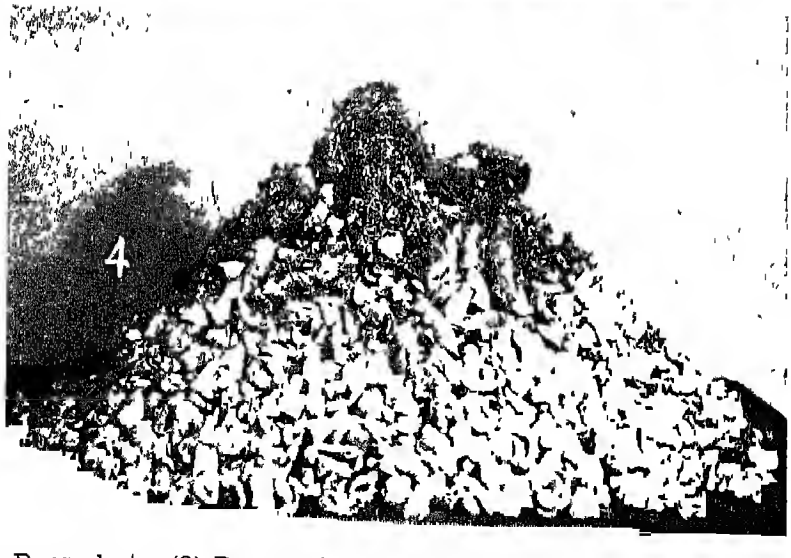
TABLE II

Name of the Plant.	Petr. Ether. %	Ether. %	Chloro-form. %	Ethyl Acetate. %	Alcohol. %	Total Extract. %
D. alata ..	0.28	0.20	1.36	0.28	0.85	2.97
D. glabra ..	0.88	0.49	2.53	2.34	4.81	11.05
D. oppositifolia .	0 58	0.55	0.32	1.12	2.91	5.48
D. Wallichii ..	0.63	0.35	0.37	2.11	4.28	7.74
D bellophylla ..	0.80	0.37	0.56	2.15	10.45	14.69
D. pubera ..	0.69	1 05	0.31	1.13	4.65	7.83
D. nummularia ..	0.53	0 78	0.54	1.35	3.23	6.43
D. aculeata ..	0.66	0 32	0.28	0.92	0.86	3.04
D. esculenta ..	0 92	1 19	1.09	4.54	2.87	10.61
D. hispida (Indian) ..	0.41	0 67	0.95	1.18	0.94	4.15
D. pentaphylla	0.91	0 35	0.24	1.18	1.51	4.19
D. tomentosa	0.44	0.71	0.43	1 05	0.52	3.15
D prazeri ..	0.85	2 31	0 93	7 63	9.51	21.23
D. bulbifera (Indian) ..	0.41	0.55	0.58	1.27	1.61	4.42
D. deltoidea	1 00	1.03	1.07	3.71	21.01	27.82
D. sativa ..	0.45	1.43	0.37	1 85	0.99	5.09
Unidentified Species						
No. 1 ..	0.71	0.98	0.24	1.15	10.30	13.38
No. 2 ..	0.67	0.66	0.28	2.92	1.44	5.97
No. 3 ..	0.53	0.49	0.27	1.44	8.13	10.86

The air-dried powdered yams were extracted successively with petroleum ether (b.p. 40-60°), ether, chloroform, ethyl acetate and alcohol in an all-glass Soxhlet extractor. The solvent of each fraction was evaporated separately and the residue weighed. The results are stated in table II. The individual fractions were then tested for the presence of saponin as follows:—

PLATES OF DIOSCOREA YAMS





(1) D. esculenta, (2) D. prazeri, (3) D. bulbifera (Indian), (4) D. deltoidea.

(1) 50 mg. of the fraction was shaken vigorously with 50 c.c. of water. Saponins under these conditions produce lather with water with characteristic honey-comb structure. Unlike proteins, the lather produced by saponins is stable even when the solution is heated.

(2) A solution of 50 mg.-250 mg of the specimen in a litre of water was prepared. It was added dropwise to gold fish *(Carassius auratus)* in a two litre jar of water, five fishes being used at a time. In the presence of saponin in toxic doses the activity of the fish diminishes and it becomes difficult for the fish to maintain equilibrium due to which they turn round and gradually die.

(3) A suspension of red blood cells in normal saline was treated with a small amount of the specimen. In the presence of saponin haemolysis takes place

Isolation of the sapogenins from the various yams were carried out as follows The air-dried powdered yams were exhaustively extracted with boiling 90% alcohol in a five litre Quickfit Soxhlet extractor for 40-50 hours. The solvent was then evaporated under reduced pressure and the viscous residue extracted several times with ether The ether insoluble residue containing the saponin, if any, was hydrolysed by refluxing with alcoholic hydrochloric acid (5%) for 4-6 hours The alcohol was then evaporated on the water-bath with addition of water to keep the volume constant The aqueous solution was then filtered and the residue washed with water and dried thoroughly. It was then exhaustively extracted with ether in a Soxhlet extractor. The ethereal solution was washed with 1% sodium hydroxide solution and then with water and dried over fused calcium chloride The solution was then concentrated to a small volume and left to crystallise. The product in the folowing cases could not be purified properly or found to be mixtures of sapogenins which could not be separated into the pure constituents for want of material—*D. alata, D. glabra, D. Wallichii, D. bellophylla, D. nummularia, D. hispida, D. tomentosa, D. bulbifera, D sativa* and the unidentified species No. 3. *D. esculenta* yielded a mixture of sapogenins The product on chromatography over aluminium oxide using mixtures of benzene and petroleum ether as eluents gave diosgenin as the principal fraction in 0.17% yield based on the weight of the air-dried yams It crystallises from absolute alcohol in colourless needles, m.p. 197°, $[\alpha]_D$ —128 3°. On heating with acetic anhydride it gave an acetate, m.p. 190-191°, and with benzoyl chloride in pyridine solution it gave a benzoate, m.p. 236°. The identity was established by comparison of these products with authentic specimens obtained from Professor Carl Djerassi of Wayne University, U. S A, and Syntex S A, Mexico. Both *D. prazeri* and *D. deltoidea* gave considerable yields of sapogenins and in each case the product was found to consist of diosgenin only The yields of diosgenin in the two cases were found to be 2.1% and 3 35% respectively based on the weight of the air-dried yams. The yields of diosgenin as obtained from *D. prazeri* and *D. deltoidea* are much higher than those from the various other sources (cf. table III) as recorded in the literature. It may be further mentioned that the yams of *D. deltoidea* contain as low as 20% of moisture.

General Remarks and Conclusion

We should consider ourselves fortunate in having in this country a vast resources of medicinal plants. The therapeutic values of some of these have already been established, although the major fraction still remains unexplored. It all depends how best we can utilise them to alleviate the sufferings of our countrymen and for the general development of our country. It has been the

usual practice of almost all workers on indigenous drugs in this country to isolate the active constituents and explore the therapeutic possibilities of the

TABLE III

Name of the Plant.	Yield of Diosgenin/ 1000g. dry yams.	Name of the Plant.	Yield of Diosgenin.
Mexican Dioscorea			per 1000g. of plant
D. bulbifera	4.5g	*American Trillium*	
D. capillaris	2.2	T. declinatum	5.0g.
D. composita	3.0	T. erectum	3.0
D cyphocarpa	2.0	T. Hugeri	3.0
D. dugessi	2 0	T. ludovicianum	5.0
D. galeottiana	4.0	T recurvatum	4.0
D. grandifolia	1.5	T. simile	4.0
*D hirsuticaulis	1.5	T Vaseyi	0.4
*D hirticaulis	4 4		
D jaliscana	3.1		per 1000g. of dry plant
D lobata	5 0		
D. macrostachya	2 9	*Mexican Balanites*	
D. mexicana	3 9	B. aegyptica	5.0
D. militaris	3.3		
D. minima	2.7		per 1000g of pericarp of seed
D multinervis	2.7		
D. platycalpata	3.0		
D plumifera	4 1	*Indian Balanites*	
D. pringlei	3.5	B. Roxburgii (Dhenke and Bhide, 1951)	1.0
*D. quartenata	4.4		
D remotiflora	3.7		
D. subtomentosa	4.4		
D. testudinaria	5.0		per 1000g. of dried yams
D. ulinei	4.0		
D. urceolata	4.6	*Indian Dioscorea*	
		D. deltoidea	33.5
		D. esculenta	1 7
*undried yams used		D. prazeri	21.0

product itself. There is, however, another way of utilisation—that is even in case a product directly isolated from the plant is found to have no therapeutic value it may be converted into some other product having medicinal value by adopting semi-synthetic methods involving one or more chemical steps. Our investigation on the sapogenins of Indian Dioscorea plants provides an example of this type. Diosgenin, which can be easily isolated in large quantities from *D. prazeri* and *D. deltoidea* has no therapeutic value but it can be converted into a number of useful sex hormones and cortical hormones including cortisone. As stated above the yields of diosgenin from the yams of *D. prazeri* and *D. deltoidea* are exceptionally high as compared to those from the other sources. In the case of *D. deltoidea* which appears to be the best source for diosgenin, the moisture content of the fresh yams is also very low. It is highly desirable to take proper steps for utilisation of these two Indian plants in the best possible way.

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INVESTIGATIONS ON SOME INDIGENOUS MEDICINAL OILS

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(Communicated by B. Mukerji, F N I).

A number of non-edible oils, available in considerable quantities in this country, have been reputed to have therapeutic value, and are being used in the indigenous system of medicine since ancient times. In absence of sufficient data in regard to modern therapeutic assessment of the oils or of the active principles present in them, fuller use has not been made of these important resources, either for fat or for newer therapeutic agents. The so-called non-edible oil-bearing seeds, though available in plenty, but, for lack of organized efforts for their collection and utilization, these important raw materials at present constitute a tremendous waste. Among these minor non-drying oil-bearing seeds, which are often of uncared for growth in areas otherwise not arable for food or cash crops, *nim* (*Melia indica*), *karanja* (*Pongamia glabra* Vent.), *polang* (*Calophyllum inophyllum*) and *nageswar* (*Mesua ferrea*) are comparatively more important in view of their considerable growth and production. Owing to their strong disagreeable odour, persistent dark colour and bitter taste, due to the occurance of appreciable quantities of non-fatty constituents with the glycerides, no good quality soap can be prepared with these oils, although sporadic attempts reported to have been made time and again to use them as such in soap stock or sometimes after *ad-hoc* treatment to improve the quality in regard to colour, odour and taste. As is evident from the absence of proper utilization of these oils, these *ad-hoc* attempts have often been futile and systematic chemical investigations into the glycerides as well as the non-glyceridic components were of utmost importance for their fuller industrial exploitation on scientific and economic basis. With this end in view, a scheme of research for utilization of *nim* oil and its bitter constituents was undertaken in the C S I R Laboratories and with increasingly promising results so far obtained, the scope of the work has since been considerably extended, exploring the possibilities of utilization of other non-edible medicinal oils mentioned earlier. The results of these systematic investigations, recorded in the following paragraphs, have led to newer findings in regard to the glyceride composition of these oils, the presence of any physiologically active peculiar acid such as the so-called 'margosic acid' in *nim* oil has not been confirmed, and processes have been evolved to isolate and separate the different physiologically active constituents from the non-fatty components of the oils under very mild conditions ensuring isolation of the active principles in the state in which they may be assumed to be present in the plant body, and simultaneous purification and refining of the fixed oil rendering it suitable for various industrial uses. It is hoped that the present critical studies in the chemistry and therapeutic evaluation of the physiologically active non-fatty constituents may eventually result in developing some new medicinals.

Nim oil (*Melia indica;* N. O. *Meliaceae*)

Uses of *nim* is so extensive and well known in this sub-continent that it hardly needs any repetition to emphasise its importance. What is really fasci-

nating about this plant is that the medicaments prepared from its various parts, particularly the oil and the bark for various ailments, recorded in earlier literature of Ayurvedic and Unani system of medicine have often found favour till the present day, sometimes even by the practitioners of Allopathic therapy. Suffice it to say, in the latter part of the last century and in earlier decades of this century, many European practitioners (Dymock) in India prescribed *nim* bark decoctions for the treatment of fevers particularly of malarial type. They used the oil for treating rheumatism, scrofulous glands, leprosy and variety of obstinate skin disorders An interesting use of the oil was noted in the Madras Medical Records (Watt.) that "about an ounce of the oil is given to every woman immediately after she is delivered of a child". The oil is reported to have been used as a contraceptive as well. A preparation from *nim* oil, margosic acid (Dutt) has been reported to have been found effective in the treatment of syphilis and filariasis. *Nim* has long been included in the Indian Pharmacopia as an unofficial remedy and the oil has got a rightful place in the latest Indian Pharmacopocial List, 1946 Very recently, it has been reported (Chopra *et al*) that the oil obtained from leaves, seeds and barks of nim possesses marked anti-bacterial spectrum against gram-negative and gram-positive organisms, even against *M. Tuberculosis,* streptomycin resistant strain.

The results of earlier chemical investigations into the active constituents of *nim* led to findings of a very conflicting character, without yielding any well-defined products which could, either be employed in pharmaceutical practice, or made the subject of further chemotherapeutic studies Thus the so-called margosic acid (Roy *et al*) was shown to be a mixture of well known fatty acids (Dutt & Roy) with some bitter resinous impurities. New fatty acid (Qudrat-i-Khuda *et al*) claimed to have been isolated, have again been found to be mixtures (Child & Nathenael). From the studies in the characteristics and properties of the Nimbidin series of bitters (Siddiqui) which have since been isolated from the oil, it appears that margosopicrin (Watson *et al*) reported by some earlier authors to have been isolated in much too poor yield from the soap lye, was a degradation product of nimbidin, the major bitter constituent of the oil.

Nimbidin, obtained in an yield of about 1 to 1.5% on the wt. of the oil, is a straw coloured, water insoluble, oil or petrol ether insoluble, granular powder. It is dextrorotatory (+ 65°) and from the preliminary degradation experiments, it appears to be an ester of terpenic origin as the other crystalline bitter constituent, nimbin. It is bitter in aqueous emulsions even in dilutions of over 1 in 100,000 with the taste of fresh *nim* twigs. When nimbidin is treated with alkali under mild and controlled conditions of temperature, concentration of alkali and duration of the reaction, it yields a neutral amorphous product of ketonic character and subsequently a crystalline neutral product neo-nimbidin (222°C), a crystalline acid,—nimbidic acid (235°C), and an amorphous bitter acid—nimbidinic acid constituting the main product of hydrolysis It has also been established that this hydrolysis can occur under physiological conditions, as for instance, in contact with the salivary secretion. This is an important point to settle, because it is only such an assurance that the therapeutic use, for which the crude drug is reputed, could be applicable to the isolated active principle or any of its preparations. In fact, the preparation of water-soluble salts of nimbidinic acid, has enhanced the activity of the active principle, rendering it to be more readily absorbed in the system Thus view was being supported from the preliminary pharmacological findings in nimbidin and sodium nimbidinate when the latter was found to be much more effective (in dilution 1 in 25,000) than the former (1 in 2000) in contracting the uterus of guinea pig.

Nimbidin series of bitter constituents have since been definitely established to be the active principles of the plant in so far as they have also been isolated from the root (Mitra *et al*) and trunk (Bhattacharji *et al*) bark. Analysis (Gupta & Mitra) of the refined *nim* oil (Mitra), freed of the bitter and odorescent constituents, carried out by employing the recently developed method of low temperature crystallisation and ultra violet absorption spectrophotometry shows the common fatty acids composition of the glycerides. Thus, from the results of the investigations, so far carried out, it can be safely stated that the medicinal properties of the oil are due to the active bitter and odorescent constituents and not to the fixed oil i.e. the glycerides of the *nim* seed, unlike the chaulmoogra (*Hydnocarpus kurzii*) or the gorli seed oil (*Oncoba echinata*) having C_{16} and C_{18} constituent acids containing cyclopentanyl ring, or castor oil having the constituent ricinolein acid.

The oil left after extraction of the bitter constituents still retained a considerable part of the odoriferous constituent, a sulphur containing compound of terpenic nature, which has since been isolated and is being further studied.

Karanja oil (*Pongamia glabra* Vent: N O. *Leguminosae*)

Although 'karanjin', the main active constituent present with the oil has been reported to be useful in the treatment of leucoderma, systematic investigations do not appear to have been carried out in order to fully assess the therapeutic values of the drug. Comparatively higher acetyl value of the oil were recorded by earlier workers (Desai *et al*) and it was attributed to the presence of glyceryl-dihydroxy-stearate in the oil, but recent analysis (Mitra) carried out with the purified and refined oil, free from the non-fatty constituents, showed the absence of any dihydroxy-stearic acid in the oil and the glycerides are composed of only common fatty acids.

Poland oil (*Calophyllum inophyllum* Linn; N. O *Guttiferae*)

Calophyllum inophyllum nuts of which yield a dark blue to olive green oil of disagreeable odour and bitter taste is abundantly available throughout the Pacific coasts as well as on the East and West coasts of India. The oil is highly reputed (Kirtikar & Basu, Watt) as a cure for rheumatism Its application in cases of leprosy appears to be of considerable practice. Comparatively recently, its efficacy in the treatment of burns was reinvestigated (Sanyal) and fairly established. A number of investigations (Chuta Hata) have been carried out on the composition and characteristics of this oil of different origin and it has also been found to be a mixture of glycerides of ordinary fatty acids. Very recently, isolation (Lederer *et al*) of two crystalline products, a lactone, calophyllolide, $C_{25}H_{22}O_5$ m.p. 58-60°C., and an acid, calophyllic acid, $C_{25}H_{24}O_6$, m.p 205-15°C, $[\alpha]_D^{18}$—58, from the defatted kernel, has been reported. It is interesting to note that on preliminary examination, these substances have been found to be tuberculostatic. Chemical examination (Mitra) of a sample of this oil from Orissa was undertaken in the National Chemical Laboratory with a view to its industrial utilization. During the preliminary investigations, calophyllic acid as well as a new crystalline bitter, provisionally named as inophyllin, $C_{21}H_{32}O_4$, m.p. 105°C (yield 0.3% on the wt. of the oil) have been isolated from the non-fatty constituents (alcoholic extractive) of the oil Inophyllin is laevorotatory, $[\alpha]_D^{32}$—94° and shows ultraviolet absorption maxima at 315, 230, 270 and 287 mμ.

Nageswar oil (*Mesua ferrea* Linn; N. O *Guttiferae*)

Mesua ferrea nuts, commonly known as nagkesar or nageswar which are extensively available in Assam as well as in Western Ghats, yield a reddish brown bitter oil, 70% on the wt. of the kernels. The oil is reported (Kirtikar

& Basu, Watt) to be useful as an embrocation in rheumatism, as well as in the treatment of itches. A number of investigations (Hilditch & Dhingra; Hooper; Grimme, Chatterjee & Gupta, Gupta) have been carried out with this oil with a view to decolorise and refine the oil for manufacture of soap A crystalline bitter lactone, mesuol, $C_{23}H_{22}O_3$, was isolated from the solvent extracted oil (Bose *et al*). There appears to be very little work done in regard to the therapeutic evaluation of the non-fatty active constituents present with this oil. In the scheme of investigations in the non-edible oils for their industrial utilization, undertaken in the National Chemical Laboratory, work on nageswar oil (Mitra) and its non-fatty constituents is also in progress.

A close survey of the literature as well as the results of the systematic investigations so far carried out with these non-edible medicinal oils clearly indicate that the method of cold extraction of these oils with solvents like alcohol or methanol, not only ensures isolation and subsequent separation of the active principles present in appreciable quantities with these oils as non-fatty constituents, but also renders these potential fat resources suitable for industrial utilization after subsequent usual refining. As has already been indicated, *ad-hoc* treatment of these oils with a view to use them in crude soap stock does not constitute an economic basis of their utilization as industrial raw materials; moreover physiologically active constituents are destroyed which could, otherwise, be of specific curative values after thorough chemical and pharmacological studies in them. Although, the isolation and chemotherapeutic studies in new active principles constitute a long range problem of research, it is hoped that, due attention to this problem would eventually evolve some new therapeutic agents, which, when obtained as bye-products, would contribute substantially to the scheme of industrial utilization of these less explored, but important raw materials.

Summary

Results of the chemical investigations of some of the important medicinal oils such as nim (*Melia indica*), karanja (*Pongamia glabra* Vent), polang (*Calophyllum inophyllum* Linn) and nageswar (*Mesua ferrea*) have been discussed. Comparatively easy methods have been evolved to ensure isolation and separation of the physiologically active constituents present in these oils for chemotherapeutic studies. Simultaneous purification and refining of the oils were also made thus rendering the hitherto unexplored fat resources suitable for industrial utilisation. Further studies since carried out with the purified and refined oils and their accompanying non-fatty constituents, e.g. of nim and karanja, have confirmed the absence of any physiologically active new or peculiar component acids in the glycerides composition reported by earlier authors, and preliminary pharmacological investigations of certain active bitter constituents isolated in pure state from these oils have shown promising results.

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PHYSOCHLAINA PROEALTA (DON) MIERS

(*A new Source of Atropine Group of Alkaloids*)

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Belladonna, *Hyoscyamus muticus* and *Duboisia myoporoides* form the chief source of atropine group of alkaloids. Belladonna containing 0·4—0·6 per cent. of alkaloids grows in a state of nature in the forests of Kashmir at altitudes of over 7,000 feet above sea level. It is also cultivated in Kashmir but its supplies are limited and insufficient for the growing needs of pharmaceutical industry in India *Hyoscyamus muticus* contains about 1 per cent. of alkaloids and grows wild in Egypt and Sudan. *Duboisia myoporoides* and *D. leichhardtii* are plants which are grown in Australia Their leaves contain 0·6 to 3.1 per cent. of alkaloids mainly hyoscyamine and hyoscine in high concentration. There is a steady bulk supply of leaves from this source for the manufacture of atropine and related alkaloids. Atropine is, however, not [illegible] in India due to short supply of raw materials.

During [illegible] survey and search for new medicinal plants a promising [illegible] was discovered in the inner dry valleys of the Himalayas i.e. Ladakh. This plant is found in abundance in this area and can form a good source for the manufacture of atropine group of alkaloids The plant has been identified as *Physochlaina proealta* (Don) Miers and belongs to the Solanaceae family Preliminary studies have been carried out in the Drug Researeh Laboratory and more detailed work, necessary for its commereial exploitation, is now in hand

Distribution and availability.—It is reported that the plant grows wild at altitudes of 9,000 to 16,000 ft above the sea level, particularly all over Ladakh District, Leh, Kargil and Skardu areas It is also reported to be growing in Sinkiang (China) bordering on the Ladakh District.

It is a hardy perennial which emerges from the ground soon after the snow melts in May-June chiefly on the warmer aspects of the hills. The leaves are almost purple in colour, the growth is very quick and the plants attain a good height in a short time. The inflorescence appears in tufts with erect sub-campanulate greenish yellow flowers a little later.

The annual output of the dry leaf from the whole of Ladakh District, which comprises about 38,000 square miles, may be in the neighbourhood of about 50,000 lbs. Since the area is mountainous and population very sparse a sustained yield of about 20,000 to 25,000 lbs. of dry leaves could be easily collected annually at about Rs 40-50 per maund (collection charges). The transport charges may well amount to Rs 10/- and Rs 12/- per md. from Ladakh and Kargil to Srinagar. These places are situated at a distance of 240 and 123 miles respectively from Srinagar, and the transport is usually by ponies only.

The plant is reported to be easily cultivated at suitable places in Ladakh and the annual output could be increased according to demand. It has been reported that the alkaloidal percentage of the plant varies from one locality to the other depending on the aspect, altitude and soil. It is also believed that some plants bearing a black seed-like formation due to fungus growth are more poisonous than the normal healthy plants All these and other related problems are being investigated and results will be communicated in due course.

Local Uses.—The plant is considered poisonous to horses and ponies but not to livestock, for whom it is harvested and dried as winter fodder. In horses and ponies it causes gastro-intestinal disturbances of a severe nature which if not cured in time kills the animal Locally milk, egg or meat soup or butter is given as antidote to such animals in Ladakh.

The seeds are used as vermifuge to eradicate round worms from the intestines. In Sinkiang the seeds are used as an emetic in bilious attacks. A fungal growth, develops on the stem generally and is said to be very poisonous to all types of animals. Kirtikar and Basu (1) observed that in the hills, leaves are applied to boils. The mouth swells from their touch and the head and throat are affected when they are eaten.

Chemical and Pharmacological investigation.—Handa et al (2, 3, 4) investigated the plant and observed that the dried leaves contain 1.02 per cent. of total alkaloids, 80 per cent of which is hyoscyamine; the roots contain 0.6 per cent of alkaloids mainly hyoscyamine. The leaves on further examination were found to contain 0.01 per cent. of hyoscine and fairly large amount of chloride, nitrate and sulphate of potassium. The presence of these alkaloids was also confirmed by biological experiments.

Cultivation.—The region of Ladakh is remote, mountainous and difficult to approach The collection and transport charges make it uneconomical for purposes of exploitation of this plant. Seeds of this plant received through the courtesy of the Forest Officer, Ladakh, were sown in Srinagar (5,000 ft.) to see the possibility of its propagation in the Kashmir valley. Seeds were sown in spring in well prepared beds and they germinated in about two weeks' time The plant did not attain the full size of its natural habitat and the growth was rather stunted The analytical results of the leaves collected in 1951-52 from these plants are given below and compared with the leaves obtained from Ladakh.

Further studies on this plant are in progress. Attempts are being made to cultivate the plant on a large scale in the Drug Farm at Yarikah. The seeds have sprouted and the plants are showing good growth. Pharmacognostic studies are also being carried on and the result will be declared in due course

Alkaloidal content in Physochlaina procalta cultivated in Srinagar.

	Percentage of alkaloids.
Leaves collected from first year plantations from Srinagar	0.16—0 24
Second year plantations	0.74
Leaves collected from plants growing wild in Ladakh	1 02

It would be observed from the above data that the alkaloidal percentage in *Physochlaina procalta* cultivated in Srinagar (5,000 ft) is low as compared to its original habitat in Leh (1.02%) but the percentage of active principles tends to rise with the maturity of the plant. It is possible that by improved methods of cultivation, contents may be increased even at lower altitude.

SUMMARY

A plant *Physochlaina procalta* (Don) Miers. has been discovered in dry inner valleys of the Himalayas in Ladakh at altitudes of over 10,000 ft. which contains 1.0 per cent. or more of atropine group of alkaloids. Attempts are being made to utilise this plant for production of atropine group of alkaloids on commercial scale. Experimental cultivation of this plant is also being tried.

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CHEMICAL EXAMINATION OF *GLORIOSA SUPERBA* LINN.

PART I.—CONSTITUENTS OF TENDER TUBERS

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(Communicated by B. Mukerji, F.N.I.).

The isolation of colchicine and a new alkaloid gloriosine from the fresh tubers of *Gloriosa superba*, was reported in a previous communication (Subbaratnam, 1952). As the plant is widely distributed throughout India and constitutes a potential source of colchicine and in view of the increasing interest in recent years in colchicine and related substances, it was considered desirable to undertake an extensive examination of the seeds and also the tubers of *G. superba*, collected at various stages of plant growth. Besides, these investigations would be helpful for the collection of data to explain the structural relationship and mechanism of biogenesis (Robinson 1950; Bellcau 1953) of naturally occuring tropolones

Accordingly, arrangements were made for the collection of the seeds and tubers of the plant from various parts of India through authentic sources.

The present investigation describes the isolation and characterization of various constituents from tender tubers in early stage of development, collected during the months of June, July and beginning of August, 1952, from the minor forest area, about 15 miles from the Laboratory, with the kind assistance of Dr. P. C. Joshi, the then Economic Botanist, National Chemical Laboratory and the gardeners of the N. C. L. Farm

For the isolation of the alkaloids, the latest method of Santavy and Reichstein (1952) was followed in order to separate any basic constituent. However, the total alkaloid obtained from these immature tubers, (yield 0.006% on the weight of dry tubers as against 0.1% alkaloid obtained earlier from dry mature tubers) fractionated through chromatography on neutral alumina was found to contain neither colchicine nor gloriosine but a new alkaloid m.p. 258-60°C (dec.) (yield, 0.015% on the weight of dry tubers). Attempts to obtain the residual alkaloids in crystalline form have not yet fructified and the findings will be reported in a subsequent communication.

Studies in the characterization of the alkaloid m.p. 258-60°C (dec.) carried out so far indicate it to be similar to "substance B" m.p. 264-67°C, isolated by Santavy *et al Santavy and Reichstein* (1950) Santavy and Bartek (1952) from *C. autumnale* earlier and more recently from species of *Gloriosa* collected in Czechoslovakia and Holland. The U.V. absorption curve of the alkaloid in ethanolic solution is similar to that of substance "13" and the accompanying table (Table I) illustrates the comparative properties of the alkaloid and its colchicic acid (II) derivative obtained by reaction with sodium methoxide (Santavy and Reichstein 1950; Santavy 1948) with those of "Substance B" (I).

Santavy *et al* have assigned the constitutional formula, N-formyl desacetyl colchicine to "Substance B" but have not provided any evidence for the presence of the "N-formyl group" in the substance. In the [illegible] dure tests (qualitative and quantitative) for the estimat[illegible] which confirm the presence of the N-formyl group in the alkaloid, have been recorded.

TABLE I.

	Alkaloid, from tender tuber of *G. superba*	"Substance B"
Colour and form of crystals	Pale yellow prisms	Pale yellow pyramids
M.P. °C	258-60	264-67
Molecular formula	$C_{21}H_{23}O_6N$	$C_{21}H_{23}O_6N$
Ziesel Reaction	+	+
Colour reaction with conc. H_2SO_4	Yellow	Yellow
Colchicic acid derivative	m.p. 229-30°C	m.p. 222-25°C
U.V. absorption maxima	λ244 mμ—log (-=4.42 and λ350 mμ—log (-=4.2	λ247 mμ—Log (-=4.51 and λ350 mμ—log (-=4.27

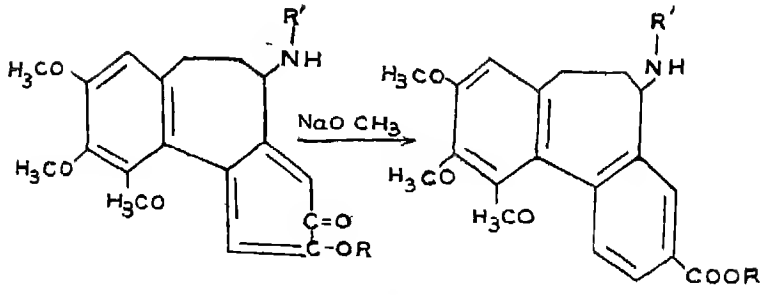

FIG. 1.

Substance B, R=CH_3; R′=HCO — Colchicic acid m.p. 222-25°C. from "substance B" R=H; R′=HCO

Colchicine, R=CH_3, R′=CH_3CO — Colchicic acid m.p. 262-66°C. from colchicins R=H, R′=CH_3CO.

Of the other constituents of these tender tubers, the isolation of γ-resorcylic acid monomethyl ether m.p. 135°C (yield. 0.04% on the weight of dry tubers) may be mentioned which was also obtained in the earlier work on mature tubers (Subbaratnam 1952) but in smaller quantity. Clewer, Green and Tutin (1915) reported first the presence of this acid in the tubers of *G. superba* and so far the occurance of this acid in no other natural product including the *Colchicum* species is known. This is of particular interest in so far as recent clinical studies carried out by Reid *et al* (1951) with a view to correlate the pharmacological action of salicylic acid with chelation in structure have established the anti-rheumatic properties of the mono sodium salt of γ-resorcylic acid, a compound with increased chelate structure, and colchicine, colchicine salicylate and extracts of *C. autumnale* as well have been occasionally used for treatment of gout and rheumatism.

EXPERIMENTAL PROCEDURE.

The fresh tender tubers (40 k.g) washed free from extraneous material were sliced into thin pieces and mashed in porcelain-ware. The pulpy material was extracted with alcohol (85%, 15 litres) by maceration and percolation at room-temperature for a period of three weeks. After collecting the percolates, the residual extract from the drug was obtained by pressing in a filter-press The marc, dried in the shade and powdered (4 k g.) was twice percolated with alcohol. The combined extracts (43 litres) were strained through thin muslin cloth and concentrated under reduced pressure at a temperature not exceeding 45°C till all the alcohol was removed. To the thick brown syrupy concentrate (2 k.g.) which was found to be acidic (pH—4.49) crushed ice and dilute hydrochloric acid were added with stirring till the resultant solution was strongly acidic (pH. 2-3). The acid solution (A) was extracted with a mixture of ether and petroleum ether (9·1, 6 extractions, 1 litre each). The ethereal solutions were concentrated to a small volume (200 c c.) and washed in turn with distilled water (20 c.c.), dilute solution of sodium hydroxide (5% 20 c c. twice) and water (20 c.c.). The remaining ether solution after drying over sodium sulphate and removal of solvent yielded a residue (1 g.) containing fatty matter and was not worked up further. The aqueous alkaline washings of the ethereal solution were cooled in ice, acidified with dilute hydro-

chloric acid and extracted with ether (30 c.c., thrice) and then with ethyl acetate (20 c.c. twice). The ether and ethyl acetate solutions were washed with water, dried over sodium sulphate and concentrated when a semi-crystalline product separated. On crystallisation from a mixture of alcohol and ether and finally from methanol (charcoal) most of it was obtained in wooly clusters and long needles, m.p. 135°C (yield 1.5 g.) With alcoholic ferric chloride it gave a violet colour as reported by Clewer, Green and Tutin; Found: C, 56.98; H, 4.66; OCH_3, 18.4; γ-resorcylic acid mono-methyl ether requires: C, 57.1; H, 4.8 and OCH_3 18.2 per cent.

The main aqueous acidic solution (A) freed from ether soluble acidic and fatty components was cooled in ice, basified by the addition of dilute ammonia and extracted with ether (three extractions, 500 c.c. each). After separation of the ethereal layers (E), the solution was rendered strongly acidic by the addition of dilute hydrochloric acid (pH 2-3) and repeatedly extracted with chloroform (6 extractions, 1 litre each). The chloroform solutions (C_1) were collected and the remaining aqueous acidic solution was made alkaline by the addition of a cold solution of dilute ammonia and extracted with chloroform (4 extractions, 700 c.c. each). The chloroform extracts (C_2) were separated and the residual aqueous solution which did not contain any alkaloid was rejected.

The ether and chloroform extracts (E, C_1 and C_2) were washed with small quantities of water, dried over anhydrous sodium sulphate and concentrated under diminished pressure, the last traces of solvent being removed in vacuum desicator. The gummy residue (0.15 g.) from the ether fraction contained some alkaloid indicated by the precipitate formed with Dragendorff's reagent but did not yield any crystalline product. The residue (2.0 g.) from the first chloroform extract (C_1) was taken up in benzene (150 c.c.) and passed through a column of neutral aluminia (60 g.) and eluted in succession with benzene, benzene+ether, ether, ether+chloroform, chloroform, chloroform+methanol and finally methanol (150 c.c. each of the eluant).

From the various fractions solvent was removed under reduced pressure. The residues from ether+chloroform (2:1 and 1:1) fractions on repeated crystallisation from a mixture of ethyl acetate and ether and finally from methanol yielded a crystalline product (0.6 g.) in pale yellow prisms which sublimed slightly between 235-240°C and melted at 258-60°C (dec.).

The residue (0.3 g.) from the second chloroform extract (C_2) was similarly chromatographed and again the ether+chloroform eluants yielded a very small [illegible] the same crystalline product m.p. 258-60°C (dec.). The residual alkaloids in the various fractions from the above working were taken together and again chromatographed on alumina, but did not yield any definite crystalline product.

Characterization of the Crystalline Alkaloid m.p. 258-60°C (dec.).

On subjecting to Ziesel reaction, the alkaloid gave a dark green colour with ferric chloride. It dissolves in conc H_2SO_4 with a deep yellow colour and its solubilities are similar to those of "Substance B" and colchicine and the extinction curve in U.V. (Fig. I) of the alkaloid (6.92 mg. in 1 litre of absolute alcohol) is also similar to that of "Substance B"; found after drying in vacuo at 100°C for 4 hours over phosphorus pentoxide: C, 65.37; H, 5.65 N, 3.65; OCH_3, 31.71; $C_{21}H_{23}O_6$ requires C, 65.44; H, 6.02; N, 3.64 and OCH_3 (for four methoxy) 32.2 per cent.

Preparation of Colchicic Acid.

To the alkaloid (100 mg.) dissolved in absolute methanol (3 c c.) in a R.B. flask (fitted with a ground glass joint condenser carrying calcium chloride guard tube) sodium methylate (5 c.c., prepared by dissolving 0.1 g. Na in 5 c c methanol) was added and the contents were heated slowly to boiling and then refluxed for 45 minutes. The solvent was then distilled off under reduced pressure from the reaction mixture and the residue was taken up in small quantity of water and acidified with dilute HCl when a white precipitate was obtained. It was filtered and washed with small quantities of distilled water, dried over porous plate in vacuum desicator and then crystallised thrice from ethyl acetate (charcoal). The product was obtained as colourless needles m.p. 224-26°C; found after drying in vacuo over P_2O_5: C, 64 18; H, 5 55; $C_{20}H_{21}O_6N$ requires C, 64.69 and H, 5.66 per cent.

Estimation of Formic Acid.

The steam distillate containing volatile acidic hydrolysis product obtained by following the micro-method (Niederl and Niederl 1942) for the determination of acyl groups, reduced acidified permanganate and gave a positive test for formic acid when subjected to the formaldehyde—milk test (Bose and Siddiqui, 1945); found formic acid, 10.99; $C_{21}H_{23}O_6N$ requires (for one formyl group) 11.95 per cent

Summary

The alkaloidal content of the young, tender tubers has been found to be low, about one-half of that obtained from mature tubers The total alkaloid dies not contain colchicine or gloriosine but consists predominantly of a crystalline alkaloid m.p. 258-60°C similar to "substance B" m.p. 264-67°C reported to have been isolated by Santavy et al.

Acknowledgements

The author takes this opportunity of expressing his sincere thanks to Prof. R C Shah for his keen interest and helpful suggestions in the course of the work. He is also thankful to Mr V. S. Pansare, Dr. G D. Shah and Dr. D. Subba Rao for the micro analyses recorded in this paper.

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THE CHEMISTRY AND PHARMACOLOGY OF *AJMALINE*, *RAUWOLFININE* AND *SERPENTINE*.

PART I: CHEMISTRY

by A. CHATTERJEE *and* S. BOSE, *Department of Pure Chemistry, University College of Science, Calcutta.*

(Communicated by B. Mukerji, F.N.I.).

Rauwolfia serpentina, Benth. (*Fam. Apocynaceae*) has received much attention as a drug for its good vasodilating properties and has thus secured for it a prominent place in modern medicine (2,7-12,21,22). Systematic chemical researches on *Rauwolfia serpentina* have been undertaken for nearly two decades The presence of five alkaloids in this species was first reported by Siddiqui and Siddiqui (27-29). These alkaloids have been classified into two distinct groups viz, ajmaline group (a group of weak bases containing ajmaline m.p. 159-160°, $C_{20}H_{26}N_2O_2$, ajmalinine, m.p. 180°, $C_{20}H_{26}O_3N_2$. 1 5 H_2O and ajmalicine, m.p. 250-52°) and serpentine group (a group of strong bases containing serpentine, m.p. 158°, $C_{20}H_{20}O_3N_2$ and serpentinine, m.p. 263-65°). From the same raw material van Itallie and Steenhauer (15) have isolated only three alkaloids namely rauwolfinine, $C_{21}H_{26}O_3N_2$, m.p. 159-160° (later proved to be identical with Siddiqui's ajmaline by Robinson et al (25), and two other alkaloids designated as B, m.p. 262° and C, m.p. 177°. The alkaloids—B and C are supposed to be identical with Siddiqui's serpentinine and ajmalinine respectively. Siddiqui (30) later reported that *R. serpentina* of Dun valley variety contains two other isomers of ajmaline, viz., iso-ajmaline, m.p. 265-6° and neo-ajmaline, m.p. 205-7°, dec. The Swiss chemists Schlittler et al (26) have isolated so far from *R. serpentina* three alkaloids viz., ajmaline, serpentine and a new alkaloid reserpine, (17) m.p. 263-5°, the latter being strongly hypnotic and hypotensive (though not remarkable) whereas the present authors (4, 6) have isolated in the pure state from the same species ajmaline, serpentine and another new alkaloid-rauwolfinine, $C_{19}H_{20}N_2O_2$, m p. 235-236°.

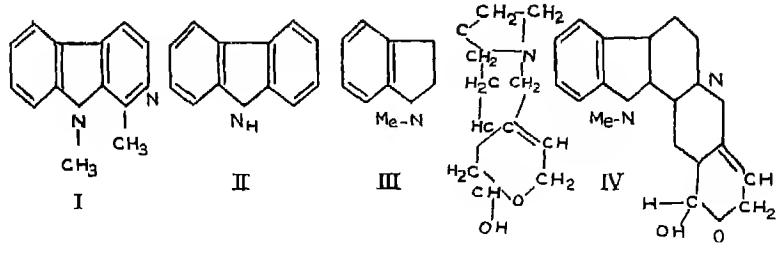
Ajmaline and rauwolfinine were precipitated out from the crude alcoholic extract of the roots of *R. serpentina* by Na_2CO_3 and the mother liquor left after the removal of the weak bases (ajmaline and rauwolfinine) was made alkaline with sodium hydroxide when serpentine separated out.

AJMALINE

Ajmaline (27-29) $C_{20}H_{26}O_2N_2.3H_2O$ m.p. 159-160° is dextrorotatory, $[\alpha]_D^{88°} = +128°$ (in chloroform). It forms a crystalline hydrochloride. When hydrated, $C_{20}H_{20}O_2N_2.HCl.2\ H_2O$, it melts at 133-4° and at 255-57° (when perfectly anhydrous). On heating at 200°, or on boiling with alcoholic potassium hydroxide, ajmaline produces an isomeric compound, isoajmaline, m.p. 265-6° which is also a natural product (30). The chemistry of ajmaline has recently been studied by Robinson et al (18). They have shown that ajmaline has strychnidine like reactions and is a monoacidic ditertiary base. The nonbasic nitrogen atom carries a methyl group. Ajmaline has an isolated double bond and has a semiacetal group although its infrared absorption spectrum shows the absence (18) of the absorption band for semi-acetal group.

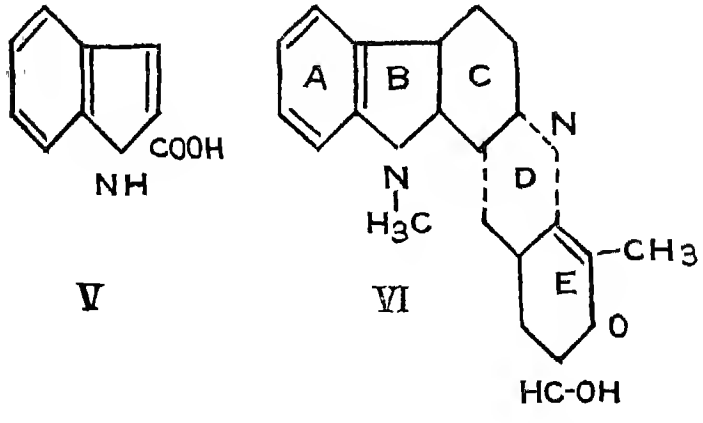
Upon distillation with soda lime and zinc dust the base produces Ind-N-methyl harman (I) and carbazole (II). From these experimental data Robinson and his collaborators have suggested either of the following hypothetical structures (III and IV) ·

FIG I.



They, however, prefer the blocked hydroindole structure (III) for ajmaline because compounds having the structure (IV) which represents a dihydroindole derivative have not yet been found to occur in nature.

The infrared absorption spectrum of ajmaline studied by the present authors (5) shows an absorption band at 5.82 μ indicating about 15-20% carbonyl in the base, an absorption band at 7 24 μ for a side chain methyl group (confirmed by Kuhn-Roth experiment), a band at 6.2—6 8 μ for a dihydroindole nucleus and an absorption band at 9 μ for an ether bridge. On fusion with alkali ajmaline produces indole-2-carboxylie acid (V), a base and a nitrogen free acid which are under investigation. On dehydrogenation with selenium the base yields Ind-N-methyl harman (I). From the collective review of these experimental data it has been proposed by Chatterjee and Bose (5) that ajmaline has the following partial structure (VI). The position of the side chain methyl group in ajmaline is either in ring B like that in physostigmine (25) or calycanthidine (16) or in ring E as that in serpentine (and thus the ring E in ajmaline will be six membered and not seven membered as postulated by Mukerji, Robinson et al (18).



RAUWOLFININE

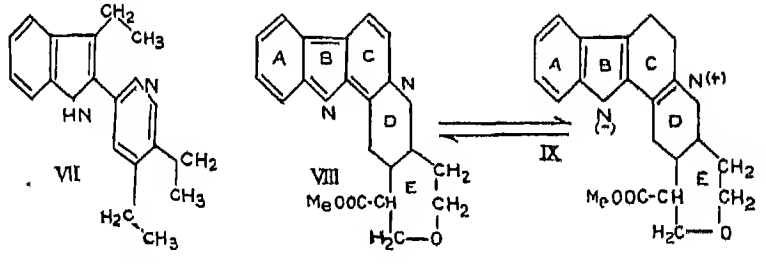
Bose (4) has reported that rauwolfinine, m.p. 235-236° dec. is optically active and laevorotatory, $[\alpha]_D^{32} = -34{,}7°$. It is free from carbonyl, methoxy

and methylene-di-oxy groups. It contains one N-methyl and a → CMe groups. The U. V. absorption spectrum shows the maxima at 249 mμ, 292 mμ and minima at 226 mμ and 272 mμ respectively. Rauwolfinine is a monoacidic base and produces a monohydrochloride, $C_{19}H_{20}O_2N_2.HCl$, m.p. 195°, dec.

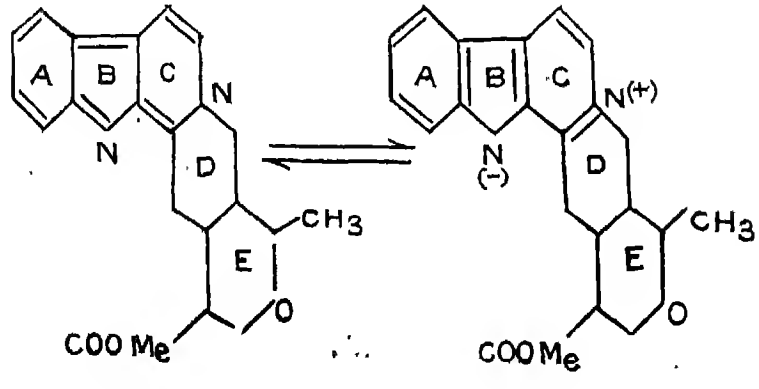
SERPENTINE

Serpentine, the strong base of *R. serpentina* forms bright yellow plates, m.p. 158°. The formula $C_{20}H_{20}N_2O_3.1.5\ H_2O$ was first assigned to serpentine by Siddiqui (27, 28). It has now been revised by Schlittler et al (26) as $C_{21}H_{22}O_3N_2$. Serpentine produces a crystalline hydrochloride, m.p. 133.5° when moist or 260°-261° when dry. The base contains two double bonds, one active hydrogen, a carbomethoxy group and no methylimino and hydroxyl groups. Serpentine is a monoacidic tertiary base. It is an indole alkaloid but the base does not contain any indole > NH group. Upon dehydrogenation with selenium serpentine produces alstyrine (VII).

Although the I.R.—absorption spectrum of serpentine shows the absence of indole > NH group, it appears in the I.R.—absorption spectrum of serpentine hydrochloride and Py-tetrahydro-serpentine. In this respect it closely resembles sempervirine (31,32). From an analogy of serpentine with sempervirine (31,32) in their properties and from various other evidences secured on physical and chemical data, Schlittler and Schwarz (26) suggest the following tautomeric structure for serpentine (VIII and IX):



Recently these structures have been revised by Bader and Schwarz (7). They have shown the presence of a → CMe group in serpentine and the side chain methyl group has been located in ring E as on dehydrogenation with selenium, serpentine produces alstyrine (VII). In the light of this new observation the structural formula of serpentine has been modified by Bader and Schwarz (1) as shown below ($X_a \rightleftarrows X_b$)



THE CHEMISTRY AND PHARMACOLOGY OF *AJMALINE*, *RAUWOLFININE* AND *SERPENTINE*.

PART II PHARMACOLOGY

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(Communicated by B. Mukerji, F.N.I).

Pharmacological investigations, using a pure alkaloid from *Rauwolfia serpentina* (R s.) were first carried out by Chopra, Gupta and Mukerji in 1932 (8). The alkaloid which these authors used was at that time considered to be identical with Siddiqui's Ajmaline (28, 29), but it was isolated independently. The substance was found to lower the blood pressure markedly, possibly by an inhibitory action on the central nervous system in addition to its peripheral vasodilator action; paralysis of the respiratory centre was also noticed.

Raymond Hamet also noted the hypotensive action of Ajmaline (23), the alkaloid used by him was obtained from Siddiqui. The main result of R. Hamet's work was the demonstration of the sympathicolytic action, not only for Ajmaline, but also for Siddiqui's Serpentine (24) and Serpentinine (20)

As regards the ajmaline used by R Hamet, doubts have to be cast on its identity with the substance used by Chopra et al in 1932, since Hamet's substance had marked inhibitory effect on the intestine, whereas the contrary was found in the investigation of Chopra et al. (8).

The situation, however, became more complicated, when Chopra and Chakravarti (10) reinvestigated the problem it was then found, that Ajmaline lowered the blood pressure only in decerebrated animals, whereas rise of arterial pressure was observed in intact anaesthetised animals Serpentine on the other hand, was hypotensive, no matter whether the medullary vasomotor centre had been destroyed or left intact. Neither Ajmaline, nor Serpentine had any sedative effect in non-anaesthetised animals; Ajmaline, moreover, was described as giving rise to convulsions.

In extension of this work, Chopra, Bose, Gupta and Chopra (12) found, that the hypnotic and sedative principles of R. s were mainly present in the alcoholic extract of R s. roots and in the total alkaloids, freed from Ajmaline, Serpentine and Serpentinine.

Siddiqui (30) has reported that the Dehra Dun variety of R s. contains two alkaloids, which differ from the alkaloids found in the Bihar variety; they were named iso- and neo-Ajmaline. Both these alkaloids appear to act predominantly depressant on the central nervous system, although an initial excitatory action was regularly seen, and they lower the blood pressure in intact, decerebrate and spinal animals (2) These alkaloids are also depressant on the intestine and therefore resemble pharmocologically more the compound used by R. Hamet (loc cit).

Recent chemical work has led to the isolation of 2 more alkaloids from R. s · Rauwolfinine (*vide* part I) and Reserpin (17) were described Pharmacological data are available only for the latter, showing that it has a marked hypnotic and hypotensive activity (3).

In view of the various discrepancies regarding the [illegible] action of some of the alkaloids from R. s., a reinvestigation has [illegible] here, using Ajmaline, Serpentine and Rauwolfinine The chemical characteristics

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of the alkaloids used are given in part I.§ The pharmacological investigation is restricted to the two different test procedures which we have found as most characteristic in previous experiments with total purified alkaloids (13, 14).

(1) Estimation of the sympathicolytic activity by testing the antagonism to the pressor effect of adrenaline and of electric stimulation of the splanchnic nerves.

(2) Assay of the central depressant action on vasomotor tone and vasomotor reflexes by administering the drug intracisternally.

Both methods have been described in details in previous publications (13, 14): for method (1), cats were used in chloralose—anaesthesia. The experiments of method (2) were performed on rhesus monkeys (5-7 kg body weight, Dial anaesthesia); the carotid sinus reflexes were elicited in these animals by clamping both common carotid arteries for 15-20 seconds.

The report is based on 12 experments of type (1) and 15 experiments of type (2). All the compounds tested were used in aqueous solution.

Results

The alkaloids Ajmaline, Serpentine and Rauwolfinine showed in the two different test procedures qualitatively the same action as they were described in our previous communications for the total purified alkaloids; (13, 14) quantitative differences were, however, noted

Ajmaline and Serpentine lowered the blood pressure in intact anaesthetised cats in doses of 1-3 mg/kg (intravenously) to a marked extent, depending on the arterial pressure to start with, they reduced the pressure also in decerebrate cats At the same time, the pressor effect of adrenaline or of electric stimulation of the splanchnic nerves was practically completely abolished; no reversal of the adrenaline pressor effect was seen, in agreement with what has been found previously for the total purified alkaloids (13). Rauwolfinine appears in this test considerably less active, doses of 3-5 mg/kg usually causing a less marked fall and only a slight reduction of the adrenaline pressor effect. It was described previously, that the adrenaline antagonism of the total purified alkaloid was competitive (13) and this is valid also for the three pure alkaloids investigated here: following a dose of 1-3 mg/kg Ajmaline or Serpentine, the adrenaline dose has to be increased 3-5 times to give the original rise of blood pressure; after 3 to 5 mg/kg Rauwolfinine, increase of the adrenaline dose only to about double was required to obtain the same effect.

It was obvious that the pure alkaloids were better tolerated by the animals than either the crude extract or the total purified alkaloids· we never observed arrest of the spontaneous respiration, when individual alkaloids in the dose range mentioned were given, whereas equipotent doses of total purified alkaloids (2-5 mg/kg) occasionally made it necessary to ventilate the animals artificially. Another difference noted was, that with either crude extract or total purified alkaloids, occasionally a transient rise of arterial pressure was noted, preceding the final fall, whereas no such rise was ever observed following the administration of the pure individual alkaloids mentioned. However the fact, that the crude extract is more potent in respect to its hypotensive and adrenolytic activity, than the total purified alkaloids (13), if compared on the basis of the alkaloid content of the former, is valid also for the pure individual alkaloids (cf. 13).

If intracisternally applied, Ajmaline and Serpentine have about the same potency in lowering the arterial pressure and in abolishing the carotid sinus reflexes· in a dose of 1-2 mg (per total animal) a fall of the arterial pressure

§An additional sample of Ajmaline was kindly supplied to us by Dr. (Mrs.) D. Chakravarty; it was used as monohydrochloride, m.p. 134°C.

to half of its original value is obtained. The effect is as long lasting as in the case of total purified alkaloids, so that it is hardly possible to wait for a complete recovery of carotid sinus reflexes or of the original arterial pressure to the height which was recorded before the administration of the drug. It was again obvious, that less interference with the spontaneous respiration is caused by these alkaloids as compared with the total purified alkaloids, since a dose of 3 mg of Ajmaline or Serpentine was tolerated whenever given, whereas all the animals, receiving the same dose of total purified alkaloids showed immediate respiratory arrest (14); even a dose of 4 and 5 mg Ajmaline (intracist.) was well tolerated, higher doses were not tried. The arterial pressure was in this case lowered to about 30 mm Hg and no further fall could be obtained if 2 to 4 mg/kg ajmaline were then injected intravenously.

Rauwolfinine regularly caused in the dose of 1 mg a deeper fall of pressure (to about $\frac{1}{2}$ of the original pressure) than Ajmaline and Serpentine and appears therefore to be somewhat more active in respect to its central action than the two other alkaloids. No difference in the duration of action of equipotent doses was observed.

Neither of the three alkaloids nor the total purified alkaloids have any specific sedative or hypnotic action, if administered intravenously to rats, doses up to 20 mg/kg of Rauwolfinine and 10 mg/kg Ajmaline and Serpentine were used. Higher doses were not tolerated: the animals died within a few minutes after the intravenous injection from respiratory paralysis, developing obviously anoxic convulsions

Conclusions

Three alkaloids from R s (Ajmaline, Serpentine and Rauwolfinine) were tested for peripheral sympathicolytic and central action on vasomotor tone and reflexes: they behave qualitatively very much alike, in so far as they lower the blood pressure whatever route of administration was chosen (intravenously or intradisternally).

It is obvious from the comparison of their activity in respect to central and peripheral (sympathicolytic) action, that these two activities do not run parallel: Rauwolfinine is comparatively weak as an adrenolytic agent, but more potent in respect to its central action than Ajmaline and Serpentine This corresponds to other data on Rauwolfia serpentina for instance Reserpin, which is obviously very highly active centrally, is completely devoid of a sympathicolytic action (3). Similar observations were also made with various fractions, which were obtained as filtrates on chromatographic separation of the crude alcoholic extract of R. s. over aluminium oxide (19).

It is also interesting to note, that neither Ajmaline nor Serpentine or Rauwolfinine exert a significant sedative or hypnotic action, although their hypotensive activity is at least partly of central origin as shown in the experiments with intracisternal application. In the case of Reserpin, however, the hypotensive action obviously is paralleled by a marked hypnotic activity (3).

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STUDIES ON THE CHEMICAL CONSTITUENTS OF THE ROOTS OF *CISSAMPELOS PAREIRA* LINN.

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(Communicated by B. Mukerji, F.N.I.).

Introduction

The classical researches of King cleared to a large extent the confusion prevailing regarding the constituents of tube and pot curare[1], used as arrow poisons by South American Indians. He isolated and characterised *d*-tubocurarine chloride, a quaternary alkaloid, from curare, and it was shown that the source of this powerful neuromuscular blocking alkaloid was *Chondodendron tomentosum,* a South American menispermaceous plant In view of the increasing importance of neuromuscular blocking agents in major surgery for obtaining muscle relaxation it was thought to be of interest to undertake an investigation of some of the Indian plants belonging to the natural order Menispermaceae.

Cissampelos pareira Linn (Hindi-Parhi, Akanadi; Kashmiri-Zakhmihayat), a climbing shrub growing throughout tropical and sub-tropical India, was selected for the present investigation. Its roots are reported[2] to be used in the indigenous systems of medicine for a variety of ailments, such as heart troubles, asthma, dysentery and in intestinal tuberculosis. Wiggers[3] isolated an amorphous, ether-soluble alkaloid, pelosine, from the roots of South American *Cissampelos pareira* and Scholtz[4] considered pelosine to be identical with bebeerine. Supplies of the air-dried roots employed in the present investigations were obtained from Kashmir and from Pilibhit, U.P.

As a result of preliminary investigations of the Kashmir roots, the isolation of two alkaloids, hayatin (Plate III, Fig. a) and hayatinin (Plate III, Fig b), a neutral substance identified as *d*-quercitol, and a sterol was reported earlier[5]. Eventually, a source of the plant was located in Pilibhit and the roots obtained from this area were also investigated The Pilibhit roots gave a lower yield (0 15%) of hayatin compared to that obtained from the Kashmir roots (0.65%), and no hayatinin or quercitol These roots, however, yielded *l*-bebeerine (0.33%) which was not found in the Kashmir roots. Samples from both areas gave an essential oil (0.2%) a fixed oil (3.4%) and a sterol.

In view of the difference in the chemical constituents of the Kashmir and the Pilibhit roots, slightly different methods were employed for the initial working up of the two lots of roots. From the Kashmir variety, free hayatin and *d*-quercitol crystallised out on keeping the alcoholic concentrate of the roots in the cold. The mother liquor was shaken with petroleum ether to remove the fatty constituents and the resinous impurities were removed from the residual concentrate by dilution with water and repeated acid-base purification. The mixture of the crude bases was finally precipitated out with sodium carbonate Hayatinin was removed from this mixture by extraction with chloroform, and the insoluble residue was worked up for hayatin. In the case of the Pilibhit roots, after the removal of the deposit of free hayatin and the fatty portion, and the acid-base treatment of the resultant concentrate, the crude alkaloids were precipitated with sodium carbonate as above. *l*-Bebeerine was isolated quantitatively from this mixture by soxhletting it with ether, in which *l*-bebeerine is soluble in the hot.

The purification of hayatin presented considerable difficulty on account of its insolubility in most of the common organic solvents and because of its tendency to resinify on heating in any of the solvents. A partial purification was, however, obtained by repeatedly dissolving crude hayatin in alcoholic hydrochloric acid and making the solution alkaline with ammonia. This gave slightly coloured crystalline hayatin, m p 298°C. (dec.), but in this process about 50% of the crude base was resinified and could not be recovered by further purification. For final purification hayatin was repeatedly crystallised from pyridine-methanol mixture, the final product melting at 303°C. (dec.).

The mother liquors, remaining after the removal of the water-insoluble bases, still gave copious precipitates with the usual alkaloidal reagents. As all attempts to extract the water-soluble bases with non-hydroxylic solvents failed, these were precipitated with Reinecke salt and the double compound is now under investigation

On the basis of the analytical data formerly available[5] a tentative formula, $C_{20}H_{21}NO_4$, was assigned to hayatin Repeated purification and analyses of the purified base and of its various derivatives, and the platinum value of the chloroplatinate, however, necessitate the changing of the formula to $C_{35}H_{36}N_2O_6$ with $C_{36}H_{38}N_2O_6$ as a very close alternative Hayatin contains two methoxy groups and two methyl groups attached to nitrogen, the tertiary nature of which is shown by its behaviour towards nitrous acid and the analytical values of the quaternary salt. Determination of active hydrogens (Zerewitnoff) indicates the presence of three hydroxyl groups in the molecule which is confirmed by the formation of a triacetyl derivative with acetic anhydride and fused sodium acetate. It does not add bromine and is not catalytically reduced, showing thereby the absence of the ethylenic linkage. With ferric chloride there is no colouration, nor does it give any tests for aldehyde, ketone or the methylene-dioxy groups On the basis of these observations the following two alternative extended formulae are now proposed for hayatin:

$$\left.\begin{matrix} C_{31}H_{21}O \\ \text{or} \\ C_{32}H_{23}O \end{matrix}\right\} \begin{matrix} (NCH_3)_2 \\ (OCH_3)_2 \\ (OH)_3 \end{matrix}$$

Hayatin is optically inactive in pyridine and in dilute hydrochloric acid, and so is its methiodide in water.

The analytical data of the methiodide (Plate III, Fig. c) and the methochloride show the presence of two and four molecules of water respectively, associated with the quaternary salts, which could not be removed at 140°C. in high vacuum. This is in keeping with the observations of Kebrle et al[6], that quaternary bases, particularly of the curare type, even on drying in high vacuum, retain varying quantities of water which may be the water of crystallisation or chemically bound.

Hayatinin has tentatively been assigned the formula $C_{30}H_{32}N_2O_9$. Since only a small quantity of the base was available for the present study, and because further quantities of the roots from Kashmir could not be obtained, a detailed study of this alkaloid was not possible.

d-Quercitol was identified through its analytical data, melting point and optical rotation. Attempts to prepare its acetate did not give any crystalline derivative, as is also reported in literature[7].

The fatty portion was worked up in the usual manner Steam distillation of this material gave a pale yellow essential oil, b.p. 155-180°C /2 mm. The residue on saponification gave a sterol, m.p. 140-41°C. from the unsaponifiable

fraction and arachidic, stearic and linoleic acids were identified in the mixture of fatty acids.

None of the constituents showed any pronounced anti-tubercular activity. Hayatin hydrochloride was inactive when tested for neuromuscular blocking effect. Since hayatin has two tertiary nitrogen atoms, it was decided to prepare and test its quaternary salts for curariform activity. The methiodide was found to possess neuromuscular blocking properties. The details of the results of pharmacological investigation of hayatin methiodide have been communicated separately for publication by Pradhan and De. Briefly, hayatin methiodide has a neuromuscular blocking potency qualitatively similar and quantitatively slightly better than that of *d*-tubocurarine chloride. Its margin of safety is also slightly better and the duration of activity of both the compounds is the same. In view of these findings it would appear that Indian *Cissampelos pareira* is a valuable drug as a source of the physiologically active neuromuscular blocking agent, hayatin methiodide.

Experimental

The powdered, air-dried roots (5,600 g) from Kashmir were repeatedly percolated with alcohol at room temperature. The total percolates (54 litres) were concentrated in vacuo to 1½ litres, and left in the cold for a few days, when quercitol (36 g) and a small quantity of hayatin (4.3 g.) crystallised out. Quercitol was separated from hayatin on the basis of the solubility of the former in water. The alcoholic mother liquor was defatted with petroleum ether and further concentrated under reduced pressure to 1 litre The petroleum ether soluble fraction was worked up for the fatty constituents in the manner described later.

The thick syrup remaining after extraction with petroleum ether was diluted with water to 3 litres when water-insoluble, dark, resinous substances separated out. The clear solution was decanted, acetic acid added to it to make up to 5% strength, and the solution thus obtained made faintly alkaline with solid sodium carbonate This was again acidified with dilute acetic acid and made faintly alkaline Repetition of this process a number of times gave a black, semi-solid mass which was insoluble in dilute acid. The clarified solution, on final basification with excess of sodium carbonate, gave a cream coloured, slimy precipitate, which was centrifuged, washed with water and dried on a porous plate. The amorphous powder thus obtained (92 g) was extracted with chloroform and the chloroform-insoluble fraction, which was found to be very sparingly soluble in other organic solvents also, was taken up in alcoholic acetic acid. The acidic solution was made faintly alkaline with ammonia and kept in the cold. Hayatin (Plate III, Fig. a) separated out as a cream coloured, micro-crystalline powder (6.5 g.) melting at 298°C. (dec.) The filtrate was again treated with ammonia when a further quantity (21.2 g.) of the crude base, melting between 280-290°C (dec.), was obtained. The final mother liquor was dark in colour and gave only resinous products.

On removal of the solvent from the chloroform soluble fraction a residue was obtained which on trituration with acetone yielded some more hayatin, m p. 283°C (dec.), yield 4.6 g. The residue left after the evaporation of the mother liquor was again taken up in a minimum quantity of chloroform and kept in the cold, when crystalline hayatinin (0 55 g ; Plate III, Fig b) was obtained. The final mother liquors were passed through a column of alumina and eluted with chloroform. Some more hayatinin (0.2 g) was obtained from the initial eluates.

A similar working of the powdered roots (3,000 g.) obtained from Pilibhit (Uttar Pradesh), gave 36 g of the crude mixture of total alkaloids. The mix-

ture was found to contain an ether soluble fraction and was therefore exhaustively soxhletted with ether. The ether solution left a crystalline residue (10 g.) which, after repeated recrystallisation from toluene, gave a crystalline base melting at 163°C. On crystallisation from methanol this base melted at 214°C. but it was reconverted into the lower melting (163°C.) form on recrystallising from toluene

After drying to constant weight at 100°C. in vacuo over P_2O_5; found C, 72 54; H, 6 6, N, 5.0 per cent, $C_{36}H_{38}N_2O_6$ requires C, 72.7; H, 6.4; N, 4.7 per cent A 1% solution of the base in ethyl alcohol showed $[\alpha]_D^{25}$—291° (l-bebeerine, $[\alpha]_D$—298°). It showed no depression in melting point when mixed with an authentic sample of *l*-bebeerine. On addition of a drop of ferric chloride to a methanolic solution of the base, the characteristic port-wine colouration as given by *l*-bebeerine, was obtained.

On working up in the manner outlined earlier the ether-insoluble portion (24 g.) gave 4.6 g. of hayatin.

Hayatin: For analytical purposes the alkaloid was crystallised several times from a mixture of pyridine-methanol (2·3) and was finally obtained in the form of colourless, prismatic rods melting at 303°C. (dec.) (Plate III, Fig. a), but the yields by this method were low (50% of the crude base). It is very sparingly soluble in alcohol, methanol, acetone, chloroform, ethyl acetate and ether and insoluble in petrol ether and water. After drying to constant weight at 140°C in vacuo over P_2O_5, found C, 72 25; H, 6.17; N, 4.7; OCH_3, 10.0; NCH_3, 9.53, active H (Zerewitnoff), 0 51 per cent; $C_{37}H_{38}N_2O_6$ requires C, 72.41, H, 6 20; N, 4.83, OCH_3 (for two), 10.7; NCH_3 (for two), 10.00; active H (for three), 0 51 per cent, $C_{36}H_{38}N_2O_6$ requires, C, 72 7; H, 6.4; N, 4.6; OCH_3 (for two) 10 47; NCH_3 (for two), 9.7; active H (for three), 0.5 percent. It is optically inactive in pyridine or in hydrochloric acid solution It does not give any colouration with ferric chloride and does not show the presence of a methylenedioxy group as tested with phloroglucinol-sulphuric acid reagent. It dissolves in concentrated sulphuric acid to a colourless solution, and with concentrated nitric acid in the cold it gives a brown colour changing to red on warming On addition of a 3% solution of bromine in chloroform to a chloroform suspension or to a glacial acetic acid solution of hayatin, bromine was found to be in excess after the addition of only a few drops of the solution. Hayatin was recovered unchanged on shaking it for 6 hours with platinum black in glacial acetic acid, in an atmosphere of hydrogen. The base went into solution on warming with 10% methanolic potassium hydroxide. The solution was refluxed for four hours on the water bath, diluted with water and treated with carbon dioxide. The resultant precipitate melted at 298°C. (dec.) and gave no depression in melting point on admixture with hayatin.

Hayatin hydrochloride was prepared by dissolving the base in methanolic hydrochloric acid and precipitation with ether when it is obtained as a straw coloured, micro-crystalline powder. On crystallisation from a mixture of methanol and ether, it separated out in the form of prismatic rods melting at 286°C. (dec.). It is very soluble in alcohol, methanol and water and insoluble in ether, ethyl acetate and petroleum ether. After drying to constant weight at 100°C. in vacuo over P_2O_5; found Cl, 10 70 per cent; $C_{36}H_{36}N_2O_6.2HCl$ requires Cl, 10.87 per cent; $C_{36}H_{38}N_2O_6.2HCl$ requires Cl, 10 64 per cent.

A 1% solution of the hydrochloride in water was optically inactive

Hayatin picrate was prepared by the addition of aqueous picric acid to a solution of the base hydrochloride in water It separated out in the form of clusters of yellow needles, which after recrystallisation from methanol melted at 234-35°C. (dec.), after shrinking 217-20°C.

Hayatin chloroplatinate was obtained in the form of a buff coloured, micro-crystalline powder by the addition of a 2% solution of platinic chloride to an aqueous solution of the base hydrochloride. The air-dried sample sinters at 285°C. After drying to constant weight at 100°C. in vacuo over P_2O_5, found Pt, 19 96 per cent, $C_{35}H_{36}N_2O_6.H_2PtCl_6$ requires Pt, 19.7, $C_{36}H_{38}N_2O_6.H_2PtCl_6$ requires Pt, 19.42 per cent.

Hayatin aurichloride separated out in the form of a light brown micro-crystalline powder when a 1% solution of gold chloride was added to an aqueous solution of the base hydrochloride. The air-dried sample shrinks and darkens at 190-95°C. and there is no further change up to 320°C.

Hayatin methiodide was prepared by heating a methanolic suspension of the base with excess of methyl iodide in a sealed tube at 100°C. for five minutes. On keeping for some time about 50% of the methiodide crystallised out. It was separated by filtration, washed free of the adhering coloured impurities with acetone, and then repeatedly crystallised from a mixture of methanol and ether. The rest of the crude methiodide was recovered from the filtrate as a cream coloured, amorphous powder by precipitation with ether. The final product was obtained in the form of colourless, prismatic rods (Plate III, Fig c) assuming a cream colour on exposure to air, m p 281°C (dec) After drying to constant weight at 140°C in vacuo over P_2O_5, found C, 49.12; H, 5.32; N, 3.0; I, 28 37, OCH_3, 6 63, N-methyl, 5.6 per cent; $C_{35}H_{36}N_2O_6.2CH_3I.2H_2O$ requires C, 49 33, H, 5 11, N, 3 09; I, 28.22; OCH_3 (for 2) 6.88, N-methyl (for 4 CH_3), 6 6; $C_{36}H_{38}N_2O_6.2CH_3I.2H_2O$ requires C, 48.92; H, 5.3, N, 3.0; I, 27 2; OCH_3 (for 2), 6.6, N-methyl (for 4 CH_3), 6 4 per cent

The molecules of water associated with the methiodide are not removable even on prolonged drying in vacuum at 140°C. A saturated aqueous solution of the methiodide does not give any colouration with aqueous ferric chloride.

Hayatin methochloride was prepared by treatment of an aqueous solution of the methiodide with freshly precipitated silver chloride. After crystallisation from a mixture of methanol and ether it melted at 306°C (dec). After drying at 100°C in vacuo over P_2O_5; found Cl, 9.31 per cent; $C_{35}H_{36}N_2O_6.2CH_3Cl.4H_2O$ requires Cl, 9.42; $C_{36}H_{38}N_2O_6.2CH_3Cl.4H_2O$ requires Cl, 9 25 per cent.

Hayatin methoplatinate separated out in the form of a buff coloured powder by the addition of a 2% solution of platinic chloride to a solution of the methochloride in water The air-dried sample sinters at 285°C.

Hayatin ethiodide was prepared in the same manner as the methiodide. After repeated crystallisation from a mixture of methanol and ether the light brown, prismatic rods melt at 261-63°C. (dec.) with darkening at 250°C.

Hayatin butiodide was also prepared in a similar manner as above. After several purifications through ether and petroleum ether it was obtained from methanol by precipitation with ether, as a cream coloured powder which absorbed moisture from the air to give a bright yellow powder melting at 249-50°C. (dec.).

Hayatin methyl ether methiodide was prepared by refluxing a solution of the base in 10% methanolic caustic potash with excess of methyl iodide for 6 hours on the water bath. After evaporating off the solvent, the residue was diluted with water when a voluminous yellow precipitate was obtained. This formed a gelatinous mass on dissolving in hot water and subsequent cooling. It was finally crystallised from hot water containing a trace of potassium iodide in the form of small colourless needles which turned pale yellow on keeping. It melted at 270-72°C. (dec.) (bath preheated to 220°C.). Found OCH_3, 12.6, N-methyl 5.4 per cent; $C_{37}H_{40}N_2O_6.2CH_3I.4H_2O$ requires,

OCH_3, (for 4), 12.8; N-methyl (for 4 CH_3), 6.2, $C_{39}H_{42}N_2O_6.2CH_3I.4H_2O$ requires, OCH_3 (for 4), 12.67, N-methyl (for 4 CH_3), 6.1 per cent

Triacetyl hayatin was prepared by refluxing the base with acetic anhydride and fused sodium acetate for four hours. The reaction mixture was poured on to crushed ice, stirred till the acetic anhydride was decomposed, neutralised with sodium carbonate and extracted with ether. The ethereal solution was washed, dried over anhydrous sodium sulphate and the solvent removed. The crude product was recrystallised from alcohol when colourless, hexagonal plates melting at 183-84° were obtained. Found acetyl value, 17 6 per cent. $C_{35}H_{33}N_2O_3(O\,COCH_3)_3$ requires, acetyl value (for 3), 18.0; $C_{30}H_{35}N_2O_5(O\,COCH)_3)_3$ requires, acetyl value (for 3), 17.9 per cent.

Hayatinin is very soluble in acetone, from which it crystallises in fine colourless rods or needles (Plate III, Fig. b) and from chloroform in hexagonal plates It is soluble in alcohol and methanol, sparingly soluble in ethyl acetate and insoluble in ether and petroleum ether. On heating, it first melts with effervescence at about 165°C. then solidifies and finally melts to a clear liquid with meniscus at 235°C If the base is heated in a test tube on a low flame till it just melts and the vitreous solid obtained on cooling is subsequently crystallised from a mixture of chloroform-ethyl acetate, hexagonal plates, m.p. 235°C. (without previous effervescence) are obtained. After drying at 60°C. in vacuo over P_2O_5; found C, 63.54; H, 5 8; N, 5.1 per cent.; $C_{30}H_{32}N_2O_5$ requires C, 63.8, H, 5 67; N, 4.96 per cent.

Hayatinin hydrochloride was prepared by precipitation of an alcoholic hydrochloric acid solution of the base with ether. The precipitate so obtained was washed with ether and dried in vacuo. It melts at 274-75°C. (dec.).

Hayatinin picrate separated out on addition of alcoholic picric acid to an aqueous solution of the base hydrochloride. It crystallised from methanol in clusters of fine yellow needles which melt gradually at 242°C.

Hayatinin chloroplatinate was obtained as a buff coloured powder by adding a 2% solution of platinic chloride to an aqueous solution of the hydrochloride. It sinters at 250-55°C.

d-Quercitol: The neutral substance which crystallised out directly from the alcoholic concentrate along with free hayatin, was recrystallised from hot water (charcoal) when cubical crystals, m.p. 235°C. were obtained. The product was soluble in water, insoluble in cold alcohol and ether It was slightly sweet in taste and its aqueous solution was neutral to litmus and did not give any colouration with ferric chloride. It did not reduce Fehling's solution. It did not contain nitrogen or sulphur. After drying to constant weight at 120°C in vacuo over P_2O_5, found C, 43.84; H, 7.29 per cent.; $C_6H_{12}O_6$ requires C, 43 90, H, 7.31 per cent. It showed $[\alpha]_D$ +24 5 (c,1;H_2O). (*d*-Quercitol melts at 234°C. and has $[\alpha]_D$ +24.37)[8].

Composition of the Fatty Fraction

The petroleum ether soluble fraction was completely freed of solvent and the residue (190.4 g.) was steam distilled. The aqueous distillate (5 litres) was saturated with common salt and extracted with ether. The total ethereal extract was washed with water, dried over anhydrous sodium sulphate, filtered and freed from the solvent, when a pale orange-yellow essential oil (11.2 g., 0 2% on the weight of the air-dried roots) was obtained. It distilled at 155-80°C/2-3 mm., n^{30}=1 4665. It was strongly acidic to litmus and on keeping in the cold it gave a crystalline deposit melting at 54-55°C.

The brownish oil left after steam distillation had S.G.$_{30°}$=0.9063; n^{30}=1.4640; iodine value, 100, saponification value; 209.3. It was saponified

Fig a

Fig b

Fig c

with 20% alcoholic caustic potash, under reflux on the water bath for four hours, and the major portion of the solvent removed under reduced pressure. The pasty residue was diluted with water and repeatedly extracted with ether. The combined ethereal extracts were then washed with water, dried over anhydrous sodium sulphate and filtered. On removal of the solvent from the filtrate the unsaponifiable fraction (8.12 g.) was obtained as a brownish semi-solid mass On fractional crystallisation from alcohol, a sterol (1.2 g.), m.p. 140-41°C., $[\alpha]_D$, −42°(EtOH), was obtained, which in chloroform solution gave a red colour with concentrated sulphuric acid. After drying to constant weight in vacuo over P_2O_5, found C, 83.09, H, 13 25 per cent; $C_{29}H_{54}O$ requires C, 83 3, H, 12.9 per cent.

Its acetyl derivative prepared in the usual manner with acetic anhyride and fused sodium acetate, melts at 132°C.

The saponified oil, after removal of the unsaponifiable portion, was acidified with hydrochloric acid and extracted with petroleum ether The petroleum ether extract gave the fatty acids (113 8 g.) in the form of a reddish-yellow oil, $S.G._{30}=0\ 892$, $n_D^{30}=1.4588$, iodine value, 93.3, acid value, 196 8 The mixture of acids was separated through lead acetate (Twitchell's method). The alcohol insoluble lead salts yielded saturated acids (30% of the total), iodine value 26.28, acid value 189.0; mean mol. weight, 296.8 On repeated fractional crystallisation from methanol, arachidic acid, m.p. 76°C. and stearic acid, m.p 69°C were obtained. The alcohol soluble lead salts gave unsaturated acids (68% of the total), iodine value, 124 7; acid value, 201.6; mean mol weight, 278.0. On bromination in absolute ether, linoleic acid tetrabromide, m.p. 114°C. was formed.

Summary

The roots of *C pareira* from Kashmir gave two alkaloids, hayatin and hayatinin, and *d*-quercitol The Pilibhit (U P) roots, however, yielded hayatin and *l*-bebeerine and no hayatinin or *d*-quercitol. Both samples also gave an essential oil and a sterol. The methiodide of hayatin, the principal alkaloid, has been found to possess powerful neuromuscular blocking properties comparable to that of *d*-tubocurarine chloride

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Discussion

Prof. Werner—How far are the two quaternary nitrogen atoms in hayatin methiodide?

Dr. J. N. Ray—While it is interesting that a neuromuscular blocking agent has been synthesised from the principal alkaloid of *Cissampelos pareira*, it would be interesting to study the antitubercular property of the whole raw drug as also of *Adhatoda vasica* which is also reported to possess antitubercular properties.

Dr. M. L. Dhar—We do not know the length of the chain connecting the two quaternary nitrogen atoms and it would not be safe to speculate on this important point in the absence of a more detailed knowledge of the chemical constitution of hayatin.

With regard to the antitubercular property of *C. pareira*, one could perhaps not justifiably reject the indigenous claim on the basis of the absence of antitubercular property in the principal alkaloid isolated from this drug. As I have pointed out, the roots from Kashmir gave a fair percentage of *d*-quercitol a close relation of mositol, one of the B vitamins, which is found in considerable quantities in the tubercular bacillus. It may be recalled that the streptidine moiety of the powerful antitubercular drug streptomycin is built round an inositol residue. It is likely that the various products which we have isolated are really artefacts and, in nature, *d*-quercitol and hayatin and perhaps some other products, which may have escaped isolation in our present working, exist in the form of a loose complex and probably such a complex might possess some action against *Mycobacterium tuberculosis*. It would certainly be of interest to test the whole drug in experimental tuberculosis on a suitable animal.

CHEMICAL EXAMINATION OF *PICRORHIZA KURROOA* BENTH.

Part III.—Constitution of the Bitter Glucoside, Kutkin

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(Communicated by B. Mukerji, F.N.I.)

Kutkin, the bitter glucoside, isolated from *Picrorhiza kurrooa* by Rastogi, Sharma and Siddiqui (1) was tentatively assigned by them the I-β-D-glucosido-4-cinnamoyl vanillate structure on the basis of its acid and alkaline hydrolytic products. Subsequently Rastogi and Sen (2), in their attempts to synthesise kutkin with a view to compare the properties of the synthetic products with the natural glucoside, found that I-β-D-tetraacetyl glucosido-4-cinnamoyl vanillate was not identical with acetylkutin. It appeared, therefore, that in kutkin, cinnamic and vanillic acids were attached to different carbon atoms of the glucose molecule. The present paper deals with further studies elucidating the nature and positions of attachment of the two acids to the sugar residue.

Enzymatic hydrolysis of glycosides with almond emulsin has been extensively studied and the effects of the structure of the glycoside, pH, nature of the buffer used and of inorganic salts that may be employed during the hydro-

lysis, on the enzyme activity are fairly well known (Helferich and Appel (3), veibel and Lillelund (4), Helferich et al (5), YuHsieh and Koo (6).

After a number of preliminary experiments carried out to determine optimum conditions for emulsin hydrolysis of kutkin, it was found that a 2 percent solution of the substrate and of the enzyme at pH 5.0 in acetate buffer gives the maximum hydrolysis of 21 percent. The extent of hydrolysis was calculated by estimating the amount of free glucose through determination of optical density of the solution after treatment with 2·4-dinitrosalicylic acid reagent (Hostettler, Borel and Deuel (7).

The pale yellow hydrolysate, obtained through enzymatic hydrolysis of kutkin was concentrated in vacuo and the residue, after removal of the enzyme by precipitation with alcohol, was examined on paper partition chromatogram using BDH universal indicator adjusted to pH 9-10. It was found to contain only vanillic acid. On spraying the chromatogram with aniline phthalate two spots appeared, one (spot A) corresponding to glucose and the other (spot B) appearing as a yellowish-brown elliptical streak which was probably due to the presence of a mixture The separation of these components was attempted in butanol.ethanol:water mixture on a cellulose column which had been freed of metal ions with 8-hydroxyquinoline. The fraction corresponding to spot A was isolated and identified as glucose through paper chromatography and by the preparation of glucosazone The fraction corresponding to spot B and vanillic acid eluted out together These were separated by passing the mixture in 82 percent alcoholic solution through an Amberlite IR-4B column when the spot B fraction passed out unaffected and the column on elution with 1 percent alcoholic potash gave vanillic acid in a yield of 4 percent. Spot B fraction gave a yellow viscous residue soluble in acetone, alcohol, and ethyl acetate, and could not be further purified through solvents or chromatography over alumina. Paper chromatography of its acid or alkaline hydrolysates did not reveal the presence of vanillic and cinnamic acids or glucose It appears likely that this fraction is related to the enzyme preparation employed in the present case.

Emulsin (almond) hydrolysis is known to split specifically the β-glucosidic linkage at carbon atom 1 provided that there are no substituents on the carbon atoms 2, 3 and 4 of the glucose molecule. Introduction of a substituent at carbon atom 6, however, has been found to favour hydrolysability which becomes inappreciable only when the volume of the substituent becomes large (Helferich and coworkers (8); Pigman (9)) The fact that kutkin yields vanillic acid on emulsin hydrolysis shows that it is attached to carbon atom 1 of glucose through a β-glucosidic linkage The other aglucone, cinnamic acid should, therefore, be linked at carbon atom 6 of the sugar moiety since such linkage only would permit the β-glucosidase hydrolysability of the molecule. In view of our inability to locate cinnamic acid or cinnamoyl glucose in the hydrolytic products, it seemed of interest to employ taka-diastase for confirmation of the above conclusions.

Since the specificity of β-glucosidases, obtained from different sources, has been found to be different from that of almond emulsin (Miwa, Cheng and coworkers (10), Horikoshi (11); Takano (12), it was expected that taka-diastase hydrolysis might lead to some more interesting results than the emulsin hydrolysis Taka-diastase hydrolysate of kutkin, obtained in the manner similar to that adopted for emulsin hydrolysis, was passed through an Amberlite IR-4B column from which vanillic and cinnamic acids were obtained. Glucose was isolated from the acid free eluate.

It would be of interest to note that Kitasato (13) obtained similar results on the hydrolysis of populin by taka-diastase, whereby saligenin, benzoic acid

and glucose were obtained as products of hydrolysis. An impure product which he suspected to be 6-benzoyl glucose was also isolated by him. It thus appears that cinnamic acid and glucose in the present case are formed as a result of subsequent hydrolysis of 6-cinnamoyl glucose.

With a view to determine the nature of linkage of vanillic acid to carbon atom 1 of the glucose molecule, kutkin was methylated according to the process of Irvine and Purdie as modified by Anderson, Baker and Lock (14). The syrupy residue of methyl kutkin was hydrolysed by baryta solution and, after removal of barium hydroxide as barium carbonate, the filtrate was passed through Amberlite IR-4B column to separate the acids from the methylated sugars The acids were eluted out from the column with alcoholic caustic soda (1 percent) and the working up of the alkaline eluate gave two crystalline acids, melting at 173°C (yield 55%) and 133°C. (yield 44%) respectively. The former was identified as veratric acid and the latter as cinnamic acid Paper chromatography of the acid-free eluate indicated the presence of at least 4 methylated sugars. The separation of these components on a cellulose column could not be effectively achieved and no major fraction was isolated. The presence of veratric acid in the hydrolysate clearly indicates that vanillic acid is attached to the glucose molecule through an ester and not an ether linkage.

To obtain further evidence in support of the position of cinnamic acid at carbon atom 6 in the glucose molecule, attempts were made to tosylate kutkin in dry pyridine. The selective replacement of the primary tosyloxy group by iodine has been used by various workers as a diagnostic aid for locating the primary hydroxyls in various carbohydrate derivatives (Hudson and co-workers (15); Gardner and Lee (16); Foster, Overend and Stacey (17).

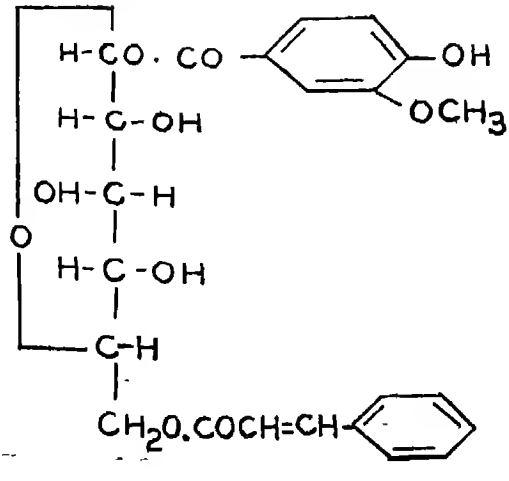
The reaction mixture, after tosylation of kutkin, gave a semi-solid residue which was finally obtained as an amorphous powder by rubbing with benzene and petrol ether. Further purification through solvents and chromatography over alumina yielded a cream coloured powder melting at 87°C. (decomp.). This product, on heating with potassium iodide in acetone solution, gave an iodine-containing compound which, after purification through chromatography, was obtained as a pale-yellow powder corresponding in its analytical data to mono-iodo-ditosylkutkin. Secondary tosyl groups, although unreactive, have been found to be replacable in certain compounds. Tipson and Cretcher (18) have shown that all the tosyloxy groups of tetratosyl erythritol are reactive under standard conditions and Hocket et al. (19) have shown that in 2.5-ditosyl 1:4-3·6-dianhydro-D-mannitol the tosyl groups are replacable. Recently Matheson and Angyal (20), after a study of a number of partly tosylated hexitols and some dianhydroditosyl hexitols, have arrived at the conclusion that "any evidence on the position of the tosyl groups derived from the iodine exchange reaction should be treated with suspicion unless the comparison is made between primary and secondary tosyl compounds of otherwise similar structural features".

It was, therefore, necessary to obtain further evidence with regard to free primary and secondary hydroxyls of the glucose moiety in kutkin. Tritylation has often been employed to detect the presence of primary hydroxyl groups in a large variety of carbohydrate derivatives (Helferich and Bigelow (21), Oldham and Rutherford (22), Levene and Tipson (23); Michael and Hasse (24), Goebal et al (25). It has been found that if trityl chloride does not react at room temperature in the absence of a significant excess of the reagent, the absence of a primary hydroxyl group can safely be assumed. Quantitative studies on the tritylation of di-isopropylidene derivatives of galactose, sorbose and glucofuranose by Hockett and coworkers (26) have demonstrated the markedly lower reaction velocity for the secondary hydroxyl group.

Kutkin was shaken with 1 mole of trityl chloride in dry pyridine solution at 30°C. The reaction mixture was treated with excess of ether to remove the unreacted trityl chloride and pyridine, and the precipitated residue gave back kutkin in the crystalline form in a yield of 29 percent The ether pyridine solution was freed of ether and diluted with water when a crystalline product, melting at 161°C., identified as triphenyl carbinol, was obtained in a quantitative yield. The failure of kutkin to give a trityl derivative and the quantitative recovery of triphenyl carbinol from the reaction mixture shows fairly conclusively the absence of a free primary hydroxyl group in the kutkin molecule

In order, therefore, to obtain a confirmation of the absence of a free primary hydroxyl in kutkin, the glucoside was treated with periodic acid at 26°C, 1 cc. aliquots were taken after suitable intervals of time and the amount of unused oxidant determined titrimetrically with 0 1N sodium arsenite A few minutes after the addition of the oxidant, the mixture became turbid and gradually an amorphous powder separated out (yield 50%). After 48 hours there was no further uptake of periodic acid and 12 6 mols. were found to have been consumed The precipitated powder gave negative tests for an aldehyde group and could not be crystallised The filtrate was not pursued further Formic acid in the reaction mixture was estimated by the method of Jones (27) and it was found that 1.66 mols. are produced during the oxidation.

Periodic acid oxidation of carbohydrate derivatives leads to the production of aldehyde and formic acid depending upon the nature of the compound. Hudson and coworkers (28) have used this reaction for the determination of the size of the lactol ring system in glycosides, and Todd et al (29) have applied it in the case of secondary and tertiary N-glycosides. Periodic acid oxidation under certain conditions, viz. at pH 7 in phosphate buffer (Lindstel) (30) or in presence of light (Head et al.) (31) has been reported to proceed in an abnormal manner leading to high consumption of the periodate. The high uptake of periodic acid during the oxidation of kutkin could be due to the presence of the vanilloyl group, since vanillic acid is known to consume three mols of periodate (Pennington and Ritter) (32) giving a dark known, amorphous power with a lower methoxyl content than the original acid The formation of formic acid in the present case is significant as this could take place only if the hydroxyls at carbon atoms 2, 3 and 4 of the glucose molecule are present in a free state. The recovery of a slightly higher percentage of formic acid could be due to the complicated and extensive degradation occurring in the molecule during the process.



On the basis of the above evidence, the structure of 6-cinnamoyl-β-D-glucosido-vanillate is proposed for kutkin.

Confirmation of this structure through synthesis is in progress.

Experimental

Emulsin hydrolysis of kutkin.

Emulsin was prepared from crushed and defatted sweet almond kernels by extraction with water, followed by precipitation of the purified aqueous extract with excess of alcohol.

A few preliminary experiments were carried out to determine the optimal conditions for hydrolysis In each case, after allowing the reaction mixture to stand at 37°C for 24 hours, 2 cc. of dinitrosalicylic acid (DNSA) reagent, prepared by the method of Hostettler, Borel and Deuel (33), were added and the test tubes heated in boiling water for 10 minutes. The yellowish-brown solution was diluted to 50 cc. and the optical density determined against a blank, which did not contain the enzyme, with a Lumetron colorimeter using a yellow green filter. The amount of glucose and hence the percentage hydrolysis was calculated by comparison with a standard glucose curve.

Table I

	Kutkin 2 mg/1 cc (cc)	Water (cc)	Emulsin 2 mg/1 cc. (cc)	pH acetic ac. sod. acetate buffer	Optical density	Glucose (mg.) Found	Hydrolysis (percent)
Effect of Enzyme Concentration:							
1.	2.0	—	1.0	4.4	0.65	0.21	14.0
2.	2 0	—	0.5	-do-	0.45	0.16	11.0
3.	2.0	—	0.25	-do-	0.15	0.08	0.5
4.	2 0	2.0	—	-do-	—	—	—
Effect of Substrate concentration:							
1.	0.5	1.5	1.0	5.0	0.0	0 0	—
2.	1.0	1.0	-do-	-do-	0.2	0.09	0.1
3.	1.5	0.5	-do-	-do-	0.55	0.18	16.0
4.	2.0	—	-do-	-do-	1.0	0.31	21 0
5.	2.0	2.0	—	-do-	—	—	—

TABLE II

Effect of Inorganic salts and pH.

Kutkin 2 mg./ 1 cc (cc)		pH Buffer	Inorganic salts 1 mg /1 cc (cc)		Emulsin 2 mg./ 1 cc. (cc)	Optical density	Glucose (mg) Found	Hydrolysis percent
1.	2	Phosphate-citrate 5 0	$KClO_3$	0.5	1	0 55	0.18	12 0
2.	2	6.0	-do-	-do-	-do-	0 55	0.18	12.0
3	2	5 0	KCNS	-do-	-do-	0 65	0.21	14.0
4	2	6 0	-do-	-do-	-do-	0.80	0 26	17.0
5.	2	Acetic ac sod. acetate 5 0	$KClO_3$	-do-	-do-	0 85	0.27	18 0
6	2	6.0	-do-	-do-	-do-	0.80	0 26	17.0
7	2	5.0	KCNS	-do-	-do-	0 90	9.28	18.0
8.	2	6 0	-do-	-do-	-do-	0 70	0.22	15.0
9	2	—	$KClO_3$	-do-	—	—	—	—
10	2	—	KCNS	-do-	—	—	—	—

Kutkin (600 mg.) was dissolved in water (300 cc.) and emulsin (300 mg.) was added to it. The solution was covered with 2 cc. of toluene to inhibit any bacterial growth and kept at 38°C for 48 hours. It was noted that some dirty white gelatinous substance separated out during the reaction which was insoluble in water and organic solvents The reaction mixture was centrifuged, the decantate concentrated to about 50 cc under reduced pressure at 50°C, diluted with alcohol (100 cc) and the precipitated emulsin removed by centrifugation. The aqueous alcoholic solution was concentrated again to 15 cc and freed of the residual emulsin by addition of alcohol (45 cc) and filtration The filtrate was concentrated to dryness under reduced pressure and the residue developed on paper for the identification of the acids and sugar in the manner given in part II (loc.cit). The paper chromatograms showed a spot for vanillic acid with BDH universal indicator (pH 9-10), one for glucose and another unidentified spot with aniline phthalate. No other acid spot could be detected

As a result of paper chromatography experiments, separation of vanillic acid and sugar components was attempted on cellulose column. A thick paste of cellulose powder (28 g) in butanol·ethanol water (4:1·5) mixture was poured in a column which was freed of metal ions with 8-hydroxyquinoline (100 mg.) in the same solvent mixture. The residue, obtained above, was dissolved in butanol.ethanol:water mixture, poured on the column and eluted with the same solvent. Thirty eluate fractions of about 5 cc. each were collected and tested for sugar and vanillic acid spots separately on the paper Fractions 5 to 9 gave spots corresponding to vanillic acid with BDH universal indicator

adjusted to pH 9-10 and another spot with aniline phthalate The latter was obtained as long, eliptical, yellowish-brown streak which seemed to consist of two substances since the upper half had a deep yellow colour (Rf, 0.85) and the lower half a brown colour (Rf, 0 68). Fractions 12-18 gave spots (Rf, 0.109) with aniline phthalate only.

Fractions 5-9 were combined and an attempt was made to separate the two components by r[illegible] over cellulose column and collecting 3 cc eluates. Th[illegible] t succeed and so all eluate fractions were mixed together, freed of the solvent and passed through a column of Amberlite IR-4B (15 g.). The eluate gave a spot with aniline phthalate but not with BDH universal indicator The Amberlite column was eluted with 82 percent alcohol containing caustic soda (1%) till the eluate did not show any spot with BDH universal indicator on development on paper. Tho total eluate (40 cc) was neutralised with hydrochloric acid (5%), concentrated and then extracted with ethyl acetate The ethyl acetate extract was washed free of acid, dried over anhydrous sodium sulphate and freed of the solvent. The residue was crystallised from hot benzene as colourless needles melting at 205°C yield 8 mg. It did not give any depression in melting point on admixture with an authentic sample of vanillic acid. The mother liquors gave a negligible residue.

The 82 percent alcoholic acid-free solution, obtained above, gave a yellow viscid residue (yield 0 23 g) which was soluble in methanol, acetone, alcohol and ethyl acetate, fairly so in water and chloroform, and insoluble in benzene and petrol ether. Its alkaline hydrolysate when developed on paper, did not give any spot for glucose, vanillic or cinnamic acid. It did not give any colouration with ferric chloride or Schiff's reagent. It could not be crystallised through any solvent and an attempt at its purification through chromatography over alumina in acetone solution and subsequent elution with acetone, acetone: alcohol (4·1), and finally with alcohol did not prove successful and no definite fraction could be isolated

Fractions 12-18, obtained above, were mixed together and gave only one spot (Rf, 0 109) corresponding to that of glucose on running on paper The combined fraction was freed of the solvent and the residue heated with phenyl hydrazine hydrochloride and sodium acetate in aqueous solution. The precipitated osazone was filtered and crystallised from alcohol, m.p. 202°C. It proved to be identical with glucosazone.

Taka-diastase hydrolysis of kutkin:

Taka-diastase used in this experiment was the produce of M/s Parke Davis & Co. prepared from *Aspergillus oryzae.*

Kutkin (200 mg.) was dissolved in water (75 cc) and taka-diastase (100 mg.) was added to it After adding a few drops of toluene, the solution was kept at 37°C. for 48 hours. The clear pale reaction mixture was passed through an Amberlite IR-4B column and washed with 82 percent alcohol till the eluate did not give any spot on paper with aniline phthalate.

The Amberlite column was eluated with 82 per cent alcohol containing caustic soda (1%, 50 cc.) The alkaline eluate was neutralized using phenolphthalein as indicator and concentrated under reduced pressure On chromatography of the concentrate on paper, both vanillic and cinnamic acids were found to be present. The concentrate was evaporated to dryness and extracted with ethyl acetate The ethyl acetate fraction was washed with water, desiccated and freed of the solvent. The residue was separated into vanillic

acid (m.p 202°C., yield 3 mg.) and cinnamic acid (m.p 133°C., yield 2 mg.) through crystallization from chloroform and then from water as described earlier.

The reaction mixture eluate and 82 per cent alcohol washings were mixed together and concentrated to 15 cc. under reduced pressure. Alcohol (40 cc) was added to the red-coloured concentrate and centrifuged. The decantate was again concentrated to 2 cc. and treated with alcohol (10 cc.) to precipitate the residual amount of the enzyme and filtered The filtrate on development on paper gave one spot only corresponding to that for glucose.

Methylation of kutkin:

Absolute methanolic solution (5 cc) of kutkin (1 1 g.), methyl iodide (2.5 cc.) and freshly precipitated, dry silver oxide (5 g) were shaken in a sealed tube for 24 hours at room temperature (30°C) Fresh quantities of methyl iodide (2 g) and silver oxide (2 g) were then added and shaking continued for 12 hours. A further quantity of methyl iodide and silver oxide were then added and the shaking continued for another 12 hours The reaction mixture was then filtered and the filtrate concentrated to dryness at reduced pressure. The pale syrupy residue was dissolved in methanol (50%, 5 cc), a saturated solution of baryta (5 cc.) added and the mixture heated on water bath for 2 hours. The reaction mixture was saturated with carbon dioxide and the precipitated barium carbonate filtered off. The residue, obtained on removal of the solvent from the filtrate, was dissolved in 82 percent alcohol and passed through a column of Amberlite IR-4B to remove the acidic components The column was washed with 82 percent alcohol till the eluate did not give a spot with aniline phthalate.

The acids were eluted with caustic potash (1%) in 82 percent alcoholic solution from the Amberlite column. The total alkaline solution was neutralized and freed of the solvent under reduced pressure. The concentrate was acidified and extracted with chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulphate and freed of the solvent. The residue gave a crystalline deposit from benzene solution. This was filtered and recrystallized from the same solvent. It melted at 173°C. (yield 0.22 g.).

After drying to constant weight at 61°C in vacuo over phosphorus pentoxide, it gave C, 59.1, H, 5.5; $C_9H_{10}O_4$ requires C, 59 3; H, 5 5 percent.

On admixture with veratric acid, it did not show any depression in the melting point The mother liquors were evaporated to dryness and crystallised from hot water when colourless needles melting at 132°C. (yield 0.14 g.) were obtained. These did not depress the melting point of cinnamic acid

The reaction mixture, obtained after the removal of the acids on the Amberlite column, and the alcoholic washings were mixed together and concentrated under reduced pressure. The residue (0 83 g), on examination on paper partition chromatogram using butanol ethanol:water:ammonia (4 1 5 0.1) as mobile phase, showed the presence of atleast 4 definite fractions, although separation was not very distinct. Development with butanol·ligroin (60·40) was also attempted but a long streak was obtained with no definite indication of any spots The residue was dissolved in butanol:ethanol water ammonia mixture and passed through a cellulose column which had previously been freed of metal ions by 8-hydroxyquinoline and 5 cc. cuts were collected. As a result of paper chromatograms of each cut, fractions 2 to 5 were mixed together and freed of the solvent in vacuo at 50°C. The residue (0.32 g.) showed two spots on paper, Rf 0.88 and 0.79, indicating it to be a mixture of

two products. Similarly fractions 6 to 8, mixed together, yielded a residue (0.01 g.) which on paper gave two spots, Rf 0.79 and 0.65. Fractions 9 to 11 on similar treatment gave a residue (0.12 g.) which showed one spot, Rf 0.41, on the paper. Fractions 12 to 16 were mixed together, solvent was removed and the residue (0.13 g.) exhibited only a faint streak on the paper.

Tosylation of kutkin:

A solution of kutkin (1 g.) and tosyl chloride (1.7 g.) in dry pyridine was allowed to stand at room temperature (22°C.) for a week. The reaction mixture was poured in excess of cold water and extracted with chloroform. The chloroform layer was washed with water, dried over anhydrous sodium sulphate and freed of the solvent. The viscous residue was rubbed with benzene to remove traces of unreacted tosyl chloride and the benzene-insoluble product was obtained as a powder on rubbing with benzene-petrol ether. It could not be crystallised and was purified by chromatography over alumina in chloroform solution. The elution was effected with chloroform, chloroform-methanol (3:1), and chloroform:methanol:pyridine (3:1:1) successively. The chloroform eluate gave a small amount of residue which was rejected; chloroform:methanol fraction, after removal of the solvent, gave a cream-coloured powder by rubbing with benzene-petrol ether. This decomposed at 87°C. with effervescence. Yield 0.7 g.

After drying to constant weight at room temperature over phosphorus pentoxide in vacuo, it gave S, 7.7. $C_{11}H_{46}O_{18}S_3$ (Tritosyl kutkin) requires S, 10.0 percent.

Chloroform-methanol-pyridine fraction on removal of the solvent in vacuo, gave a residue which also turned into a powder on rubbing with petrol ether and decomposed at 106°C. Since the yield was only 0.08 g., it was not pursued further.

Tosyl kutkin (100 mg.) was heated with sodium iodide (100 mg.) in dry acetone (15 cc.) for 2 hours in a sealed tube on a water bath. During the reaction shining plates of sodium toluenesulphonate separated out and were filtered off. The filtrate was freed of the solvent, the residue dissolved in chloroform. The chloroform solution was washed with water, desiccated and filtered. The filtrate was passed through a column of alumina which was eluted as in the previous case. The residue, from chloroform-methanol eluate, gave a yellow powder on rubbing with petrol ether, which decomposed at 88°C with effervescence. Yield 85 mg.

After drying to constant weight at room temperature in vacuo over phosphorus pentoxide, it gave S, 6.3; I, 10.8. $C_{37}H_{42}O_{16}S_2I$ requires, S, 6.8; I, 13.6 percent.

Tritylation of kutkin:

A solution of kutkin (200 mg.) and trityl chloride (120 mg.) (calc. for 1 mol., 112 mg.) in dry pyridine (3 cc.) was shaken at room temperature (28°C.) for five hours. The pyridine solution was diluted with excess of ether, cooled and the clear solution decanted off. The precipitated semi-solid mass was washed with ether, dissolved in absolute alcohol and partially precipitated with ether to remove some coloured impurities. The alcohol-ether solution was concentrated and cooled when a crystalline deposit melting 210°C. was obtained (48 mg.).

The pyridine-ether solution was completely freed of the solvent at 30°C. under reduced pressure and the residue again extracted repeatedly with ether. The ether-insoluble residue, on keeping in alcoholic solution, gave a further quantity of 210°C. melting fraction (10 mg.). The total crystalline material

(58 mg) was recrystallised from alcohol in colourless needles melting at 210-11°C. and, on admixture, did not depress the melting point of kutkin.

The ether-soluble fraction was concentrated and treated with water. The colourless crystalline material, thus obtained, was filtered and dried It melted at 161°C (100 mg.) and did not depress the melting point of triphenyl carbinol.

In order to eliminate any doubts about the unreactivity of kutkin with trityl chloride, due to its low recoverable yield from the reaction mixture in a crystalline form, kutkin (100 mg.) was treated with dry pyridine alone and then worked up in the manner given above. The residue gave only 22 mg of the original product in crystalline form and the mother liquor failed to yield any crystalline material although purification through alcohol, ether and petrol ether was also attempted.

Periodic acid oxidation of Kutkin:

After a few preliminary experments, periodic acid (1.5728 g) was added to an aqueous solution of kutkin (0.3076 g), the volume made to 10 cc. and allowed to stand at room temperature (26°C.). After a few minutes the mixture became turbid and a dirty white powder started separating out After 72 hours, 1 cc. aliquot of the mixture was removed and periodic acid estimated iodometrically by the method of Berneby (35). It consumed 0 765 cc of O.IN sodium arsenite solution, thereby showing an excess of 0 0734 g of periodic acid in the reaction mixture 1.4994 g. of the acid (equiv to 12 6 mols.) had, therefore, been utilized during the oxidation.

The yellow reaction mixture was filtered to separate the amorphous powder (0 156 g) It is insoluble in petrol ether, chloroform, ethyl acetate and methanol, and very sparingly soluble in alcohol and acetone. It does not give any colouration with Schiff's reagent nor does it reduce Fehling's solution or Tollen's reagent. The filtrate reduced Fehling's solution very readily.

In another experiment, a solution of kutkin (0 2012 g.) in water (10 cc.) was cooled, periodic acid (1.2490 g) added to it and the reaction flask, covered with a beaker wrapped with black paper from all sides to cut all the light was allowed to stand at 25°C After 24 hours 1 cc. aliquot utilized 1 525 cc , after 48 hours 1 05 cc and after 72 hours 1 0 cc of O.1N sodium arsenite solution, thereby, indicating that 0 09884 g. periodic acid was present unused in the reaction mixture 14.5 mols of periodic acid were thus consumed during the oxidation

The formic acid content of the reaction mixture was determined by the method of Jones (34) 1 cc of the solution was taken in the distillation flask used in the micro-determination of the acetyl groups by Pregl's method. 0.75 cc. of glycol was added to it and allowed to stand for an hour. 0.5 cc. of saturated aqueous sodium bisulphate solution was then added and the mixture diluted to about 6-7 cc and distilled A slow stream of oxygen was passed through the solution during the distillation which was discontinued when 50 cc. of the distillate had been collected. The distillate was yellow in colour due to the passing over of some iodine and the colour was discharged by addition of an exact amount of dilute solution of sodium thiosulphate. The colourless solution was then titrated against standard caustic soda solution using phenolphthalein as the indicator It neutralised 5.92 cc of 1.177 N/100 sodium hydroxide solution Therefore, the number of formic acid molecules liberated during the oxidation are 1.66. In another experiment, the distillate was shown to be free of cinnamic acid by the addition of a known volume of standard bromine solution in glacial acetic acid and then titration with standard sodium thiosulphate solution.

Summary

Further studies in the constitution of kutkin have been carried out As a result of emulsin and taka-diastase hydrolyses, methylation, tosylation, tritylation and periodic acid oxidation, 6-cinnamoyl-β-D-glucosido-vanillate structure has been proposed for kutkin.

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Discussion

Dr. R. C. Shah—Was fully methylated kutkin obtained in a crystalline form?

Dr. M. L. Dhar—No attempt was made to crystallise fully methylated kutkin. However, as I have already mentioned that on hydrolysis of methylated kutkin, a mixture of methylated glucoses was obtained which would indicate that it was actually a mixture. Since methylation and subsequent hydrolysis were carried out with a view to elucidate the mode of linkage of vanillic acid residue to carbon atom 1 of the sugar, it was obviously not necessary to isolate and purify the fully methylated product.

Dr. J. N Ray—I had an opportunity of paying a visit to the Central Drug Research Institute about 8 months ago when Dr. Dhar explained to me the work that was going on in connection with the structure of kutkin. I must say that their approach to this problem is very commendable It is rather unusual to obtain naturally occurring glycosides which carry aglycones on more than one carbon atom of the sugar residue. I would suggest that the ultraviolet absorption spectrum of kutkin may be compared with that of a synthetic β-glucoside carrying substituents at carbon atmos 1 and 6 of the glucose molecule.

Dr. M L. Dhar—So far as I know there are a few naturally occurring glycosides, e.g. populin, which have been shown to carry aglycone moieties on two carbon atoms of glucose. In view of the absence of cinnamoyl vanillic acid in the hydrolytic poducts of kutkin and the attachment of vanillic acid to the sugar residue through the ester linkage, leaving the phenolic hydroxyl in a free state as established by the isolation of veratcic acid from the hydrolysis of methylated kutkin, it seemed certain that the two acids could not be present on the same carbon atom. It would certainly be interesting to compare ultraviolet spectrum of kutkin with that of a known 1,6-glucoside.

INDIGENOUS DRUGS AND INSECTICIDES

by M. A. Aziz, *Department of Chemistry, Muslim University, Aligarh*

(Communicated by B. Mukerji, F N I)

Chopra[1] gives a list of 87 drugs growing in India which can be used or substituted for those in use, and quotes[2] another list of those which might profitably be investigated Some of these are used for preparation of official galenicals, some have been subjected to chemical investigation; but a thorough and systematic investigation of vegetable medicinal resources of the country has not yet been undertaken. Now, that a Central Drug Research Institute has been established at Lucknow, it may be hoped that such a scheme will be launched. This investigation requires the collaboration of botanists, chemists, pharmacologists and medical practitioners, and, finally, of industrialists to exploit its results That it is desirable, requires no stressing, when the value of imports of foreign pharmaceuticals is considered By an irony of circumstances freedom from foreign control has not given impetus to local production, but, rather, to misuse, by adulteration, and falsification. It is hoped that this deplorable state of affairs is only transitory.

The chemical investigation of plants with a view to the isolation of the physiologically active principle is no easy task. Contrary to laboratory produced chemicals, the material, with which the chemist has to deal, is of a very complex composition. Nature does not produce pure chemicals, but a mixture of closely allied substances, together with substances of different classes It is a difficult and laborious task to isolate a single chemical individual from such a mixture, and to establish its individuality Chemical literature is full of instances of this difficulty, which are illustrated by the assignment of different empirical formulae, by different investigators, to the individuals isolated from the same kind of plant.

A number of plants have been chemically examined at the Prince of Wales Chemical Laboratory at the Muslim University of Aligarh, and some of the results are enumerated below.

Ocimum brasilicum, the wild *tulsi* plant, growing round about the locality, has yielded a volatile oil. This oil has been found effective in keeping off the mosquito. It does not become indifferent to its smell by habit, as it does to the effect of D.D.T., *Cassia fistula*, the Indian laburnum, growing abundantly, in the neighbourhood, and the pulp of which is used by Hakeems as a purgative, has been found to contain frangula emodin. *Datura alba*, also growing wild locally, has been found to contain only 0.18% total alkaloids (hyoscine, and hyoscyamine) in its seeds. A sulphur compound produced from the flavanone, butin, contained in the flowers of *butea frondosa*, is awaiting pharmacological examination to ascertain its value. Pharmacological properties of flavanones have only recently (1931) attracted attention. They have been found to be diuretic, vitaminary, anthelmintic, and co-enzymatic in action The pharmacological action may be found to have been enhanced in the sulphur derivative. The oil of its seeds, which are said to possess anthelmintic property, is under investigation to ascertain if this property is due to the presence of flavanone. *Cissampelos Pareira* root, brought from a Dhak jungle in this district, was found to contain: bebeerine, 0.224%, b-bebeerine, 0.7%, isobebeerine, 0.258%, bebeerine-B, 0.16%, and chondrodine, 0.14%. The crude mixture of these alkaloids was sometimes used in medicine, but was discarded later, on account of its uncertain action, which was, probably, due to varying proportions of the constituent alkaloids. The colchicine content of the white variety of *colchicum luteum, Sooranjah talkh*, obtained from the Punjab, was found to be only 0 06%, whereas that of the black variety was 0.24%. *Atropa accuminata* roots supplied by the Drug Research Laboratories, Jammu, were found to contain 0.41% of total alkaloids, of which 3/4 could be isolated as white crystalline atropine. 0.78% santonin could be extracted in the pure colourless form from *artemesia brevifolia* (Curez variety) supplied by the same Drug Laboratories, whereas the local *artemesia* cut in the first week of August gave no santonin (cf. Chopra, *loc. cit.*). *Solanum xanthocarpum* growing wild locally gave an alkaloid which was found to induce abortion in the rabbit. A process of extraction of strychnine, free from brucine, by the use of oils, and depending on dissolution at a higher temperature, and deposition on cooling was worked out aiming at more economical production of this alkaloid.

The residues left after manufacture of tobacco were examined with a view to their utilisation as insecticide, but the work was left incomplete. Pyrethrum flowers have yielded a small quantity of pyrethrin I, the active insecticide. only.

The chemical investigations have to be supplemented by pharmacological studies if the substances so obtained are to be introduced in medicine. No agency is as yet available to carry it out, and it is highly desirable that such an agency were established as soon as possible.

References

1 Chopra, R. N., Indigenous Drugs of India (1933) Calcutta, Art Press pp 44-47.
2. *Idem ibid*, p. 26 based on Koman, Report of the Indigenous Drugs Committee Madras, (1924).

SECTION B.

PHARMACOLOGY AND CHEMOTHERAPY OF PLANT PRODUCTS

FUNGISTATIC PROPERTY OF *ACORUS CALAMUS* LINN.

by P. Y. DIGHE, G. S PENDSE *and* R. G. MODAK, *Indian Drugs Research Association Laboratory, Poona.*

(Communicated by B. Mukerji, F.N.I.)

A good deal of interest appears to have been evoked regarding the fungistatic and fungicidal properties of plants and synthetics. J Grosjean (1950) has studied the fungicidal activity of the substance in the bark of deciduous trees. Leaves are supposed to show more activity than bark in many instances. In trees of the genus Populus the distribution of inhibitors in the plant has been shown to be different and barks have been demonstrated to possess better activity than leaves.

Acorus calamus has been in use in indigenous system of medicine for various purposes, both as an external application and for its internal use. It is a common aromatic plant found in marshy places and cultivated in gardens The part used is the rhizome, which is as thick as the middle finger. It is used in medicine in a dried state and is supposed to be effective as an expectorant, emetic and is used in bronchites, stomatitis, intermittant fevers and diarrhoea in children It has been shown to be useless in the antidotal treatment of snake-bite.

These dried rhizomes were taken up for study for their fungistatic effect.

Extracts with water, alchohol, ether and petroleum ether were prepared. They were tried against the following fungi.

(1) Alternaria (2) Helminthosporium (3) Pestalozzia, (4) Piriecularia and (5) Aspergillus. First four are plant pathogens, affecting cotton, guava and rice. The fifth is a human pathogen and leads to a fairly common condition in the ear. The fungistatic property was tried by incorporating the extract in the melted potato—Dextrose-Agar medium, on which the test organisms were sown after solidification and the effects observed Appropriate controls were kept of a simple medium and medium with the same inoculum. Alcholic extract was found to be effective which was prepared as under. Twenty-five gms. of the powdered material was extracted with alchohol in a soxhlet apparatus. The Alchohol was recovered completely and the extract obtained weighed 5.357 gms This extract was semisolid and oily in nature. Chopra (1933) states that the dried rhizome yields 1.5 to 3.5 per cent of the oily extract which contains chiefly asaryl-aldehyde and a glucocide named acorine. A definite quantity of the total alchoholic extract was mixed thoroughly with water and was then added to 5 cc. of the melted medium which was inoculated after solidification The results were visually observed after every 24 hours by keeping the tubes at room temperature as well as incubating the same at 37°C. in two different sets with an idea to observe if it makes any indifference An appreciable difference could not be observed due to incubation and the results afterwards are noted at only the room temperature. Table No. 1 gives the results of the fungistatic property of the alchoholic extract of *acorus calamus*.

TABLE No. 1.

Fungistatic Property of the alcoholic extract of *Acorus calamus*.

Fungi.	24 hours. 5 10 20 30 50 (mgs.)	48 hours. 5 10 20 30 50 (mgs.)	72 hours. 5 10 20 30 50 (mgs.)	120 hours. 5 10 20 30 50 (mgs.)
1. Aspergillus ..	+ — — — —	+ 0 0 — —	+ + + — —	+ + + — —
2. Alterneria wacrospora .	+ — — — —	+ 0 0 — —	+ + + — —	+ + + — —
3. Helmenthos-porium. gosrpii ..	+ — — — —	+ 0 0 — —	+ + + — —	+ + + — —
4. Pestalozzia psidii ..	+ — — — —	+ 0 0 — —	+ + + — —	+ + + 0 —
5. Pirecularia oryzae ..	+ — — — —	+ 0 0 — —	+ + + — —	+ + + — —
6. Control medium only ..	— — — — —	— — — — —	— — — — —	— — — — —
7 Control medium & organism .	+	+	+	+

Key:— + Heavy growth. 0 Slight growth. — No growth.

It will be seen that the lowest effective quantity of the extract as observed for the period of 120 hours, is 30 mgs. of the extract for 6 c.c. of the medium (5 cc medium 1 cc. of water). This quantity is also not quite effective for pestalozzia and at the end of 120 hours slight growth is seen. With the view, therefore, to find out if the addition of small quantities of copper will be useful to enhance the action, another experiment was conducted with the addition of the 5 cc. and 1 cc. quantities of the 1% copper sulphate solution to the 30 mgs. of the extract and the effect was observed for five days. The results are given in the table No. 2.

TABLE No. 2.

Fungistatic property of Alcoholic extract of *Acorus calamus* with CuSO4.

Fungi.	24 hours 30 mg. + *0.5 cc.	24 hours 30 mg. + *1 cc.	48 hours. 30 mg. + *0.5 cc.	48 hours. 30 mg. + *1 cc.	72 hours 30 mg. + *0.5 cc.	72 hours 30 mg. + *1 cc.	5 days. 30 mg. + *0.5 cc.	5 days. 30 mg + *1 cc.
Aspergillus	0	0	0	0	0	0	0	0
Helmenthos-porium ..	0	0	0	0	0	0	0	0
Alternesia ..	0	0	0	0	0	0	0	0

Key:— (1) 0 No growth. (2) * = Copper sulphate.

TABLE No. 3.

Fungicidal Property of *Acorus calamus* on Aspergillus.

	24 hours Results					48 hours Results					53 hours Results.					72 Hours Results.					5 days Results.				
	Negative Control.	Positive Control.	75 mgs.	50 mgs.	30 mgs	Negative Control.	Positive Control.	75 mgs.	50 mgs.	30 mgs	Negative Control	Positive Control.	75 mgs.	50 mgs.	30 mgs	Negative Control.	Positive Control.	75 mgs.	50 mgs.	30 mgs.	Negative Control.	Positive Control.	75 mgs.	50 mgs.	30 mgs.
24 Hrs inoculation on plates	2	0	2	2	2	2½	0	2	2	2	2½	0	2	2	2	3	0	3	3	3	4	0	4	4	4
48 Hrs Inoculation.	1	0	1	1	1	1½	0	1	1	1	1½	0	1	1	1	2	0	2	2	2	4	0	3	3	3
53 Hrs. Inoculation.	1	0	0	0	0	1	0	1	½	½	1	0	1	½	½	1½	0	1	½	½	2½	0	1½	1½	1½
72 Hrs Inoculation.	1	0	0	0	0	1	0	0	0	0	1½	0	0	0	½	2	0	0	0	½	3	0	0	0	1½

KEY — 0=No growth.
1=very slight growth.
2=good growth
3=heavy growth.
4=luxuriant growth.

The results show that even after five days, a little addition of $CuSO_4$ to the extract has proved useful and the growth is completely absent. This aspect of the work is being followed further.

The above experiments show that the extract has an inhibitory effect on the fungi tried and it cannot be said if the organisms under trial are destroyed or killed. To understand this aspect, further experiment was undertaken on only one fungus Aspergillus, which is mentioned above as a common human pathogen affecting the ear Different concentrations of the extract were taken as before and a loopful of the organisms were inoculated in each of the concentrations. From these, inoculations were made on sterile plates of P.D.A. medium after definite intervals of time like 24 hours, 48 hours, 53 hours and 72 hours The inoculated plates were then incubated at 37°C and the results were visually observed. Thus, the organism was kept in contact with the material for different lengths of time and the results were noticed by inoculating them on sterile plates, which were incubated at 37°C. The results in each case were noted visually for the different lengths of time for a total period of five days. The appropriate controls both positive and negative were kept. The positive control consisted of 5% solution of salicylic acid, which is generally used for this ear fungus in clinical work and a negative control with the organism in contact with the same quantity of water as was used for making suspensions of the extract. The results of this experiment are given in the Table No 3.

TABLE NO 3.

It will be observed that the positive control of salicylic acid has a decidedly great effect as compared with the plant substance. The negative control shows growth from the beginning to the end, and the substance will be seen to be effective for its lethal property on the fungus after 72 hours of contact The results for 50 and 75 mgs of the extract are negative as observed for 5 days and the 30 mgs. is not a good dose for its lethal effect on the organism.

It has been a problem how the drug could be brought within the means of the masses in this country. There is a great disparity between the very low economic standards of the masses on the one hand and the cost necessary for any effective drug properly worked out and manufactured on the other, which makes any solution to the problem highly difficult. It is our plea that any crude indigenous drug which can certainly remain within the means of the masses, can be utilised as such, if its particular property and the effective mode of use is made known

SUMMARY

Fungistatic activities of *Acorus calamus* were studied and it was observed to possess a reasonable effect in inhibiting the growth of Fungi. Water, alcohol, ether and petroleum ether extracts of the drug were tried against the following Fungi:

1. Alternaria,
2. Helminthosporium,
3. Pestalozzia,
4. Piricularia and
5. Aspergillus.

The alcohol extract only was effective.

ACKNOWLEDGEMENTS

We are thankful to the Principal of the Agricultural College, Poona, for the pure cultures of plant pathogens.

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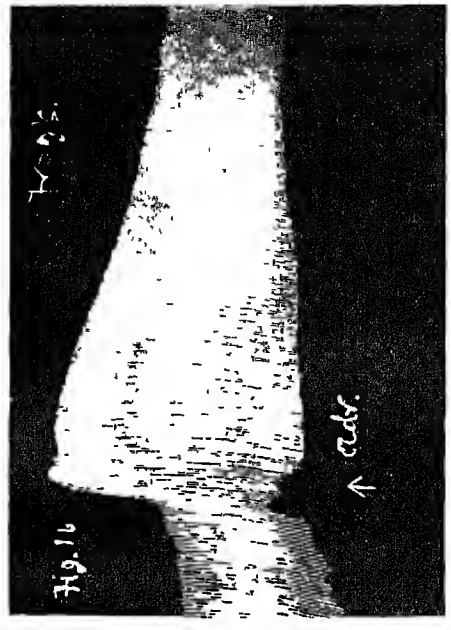


Fig. 1b.—Effect of adrenaline on the same heart.

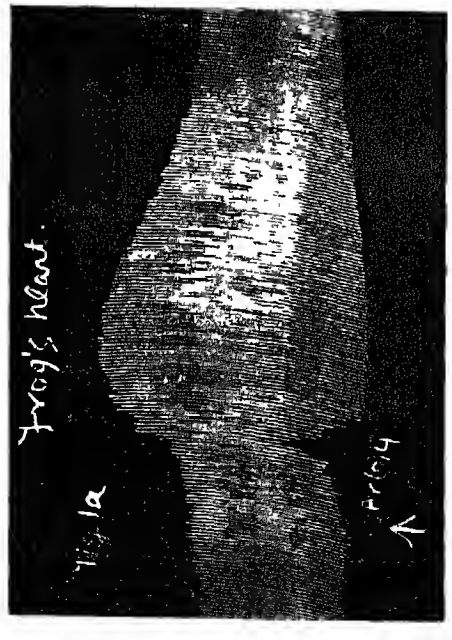
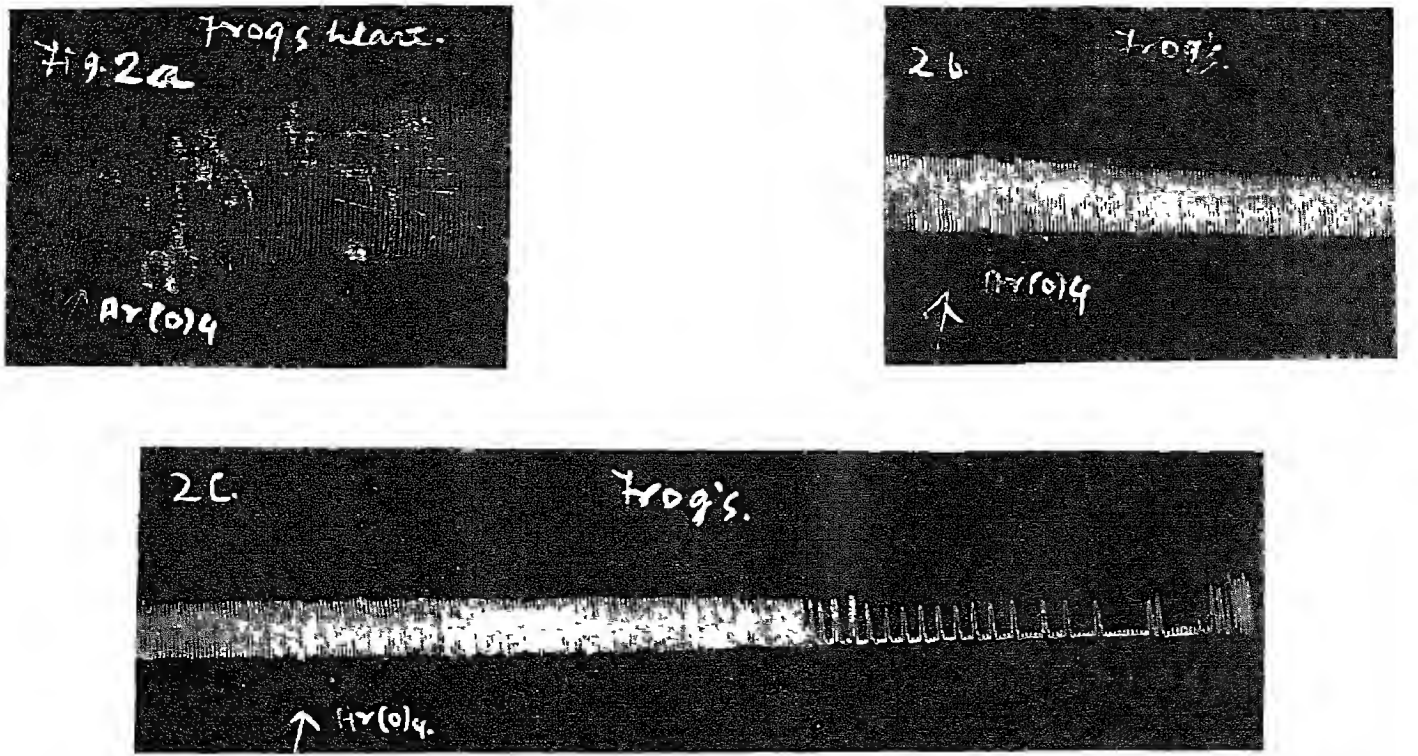


Fig. 1a.—Injected at the point of arrow; note the increase in amplitude rate little affected. C.f Fig. 1b.



Figs 2a, 2b, 2c,—note the effects of repeated injections with increasing doses—(a) increase in amplitude (b) no marked change, (c) development of irregularities

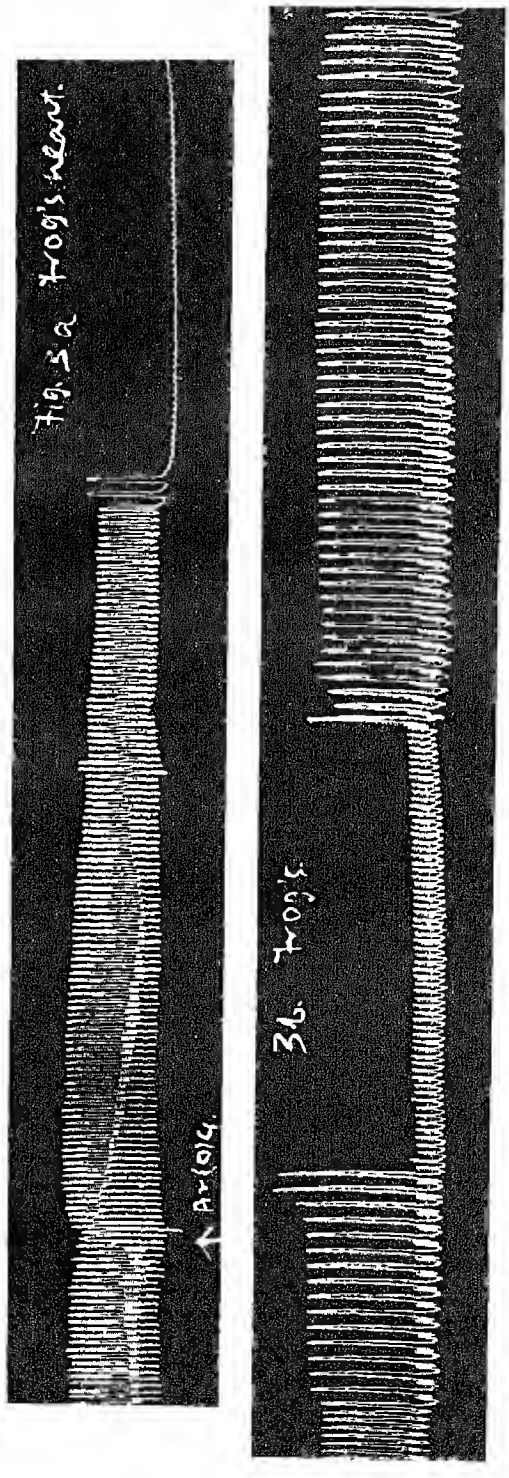


Fig. 3a.—Effect of continuous perfusion—note the complete stoppage of the ventricle
3b —Note the persistence of irregularities even after withdrawal of Ar(0)4

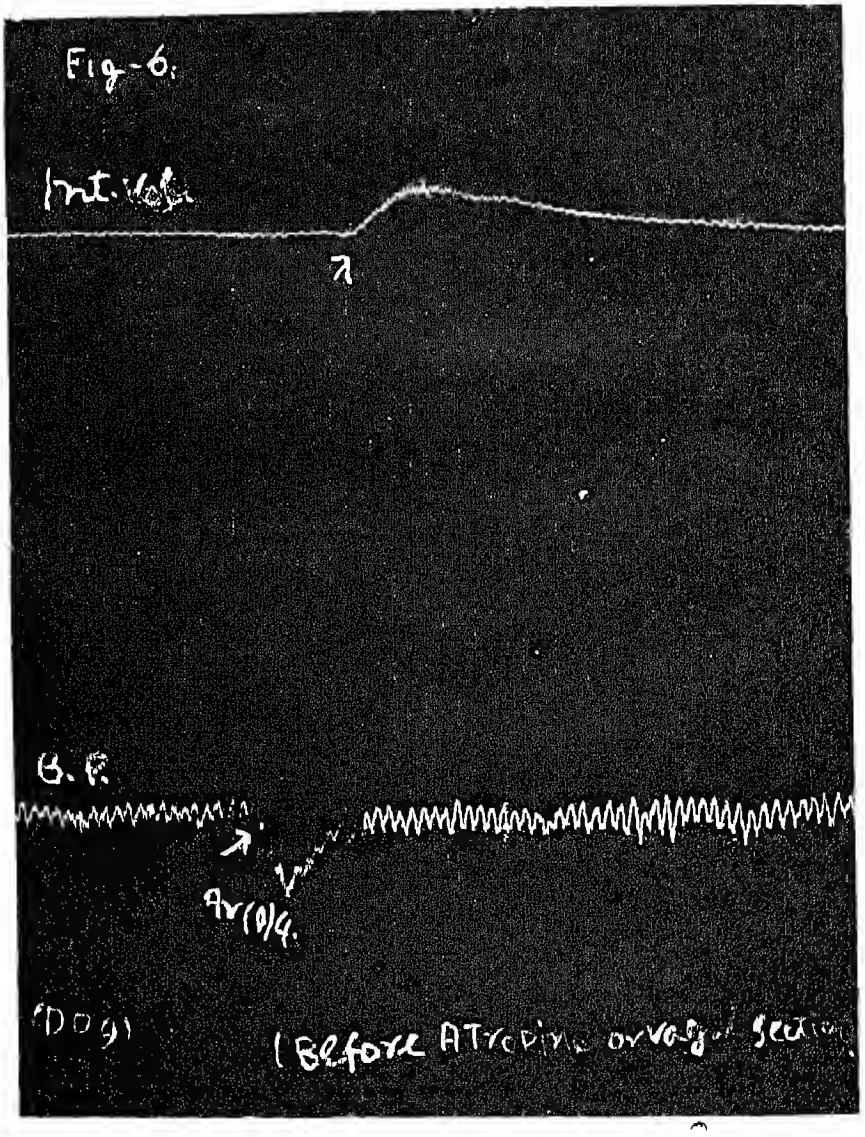


Fig 6—Injected at the point of arrow; note the immediate transient fall of B P. & increase in intestinal volume.

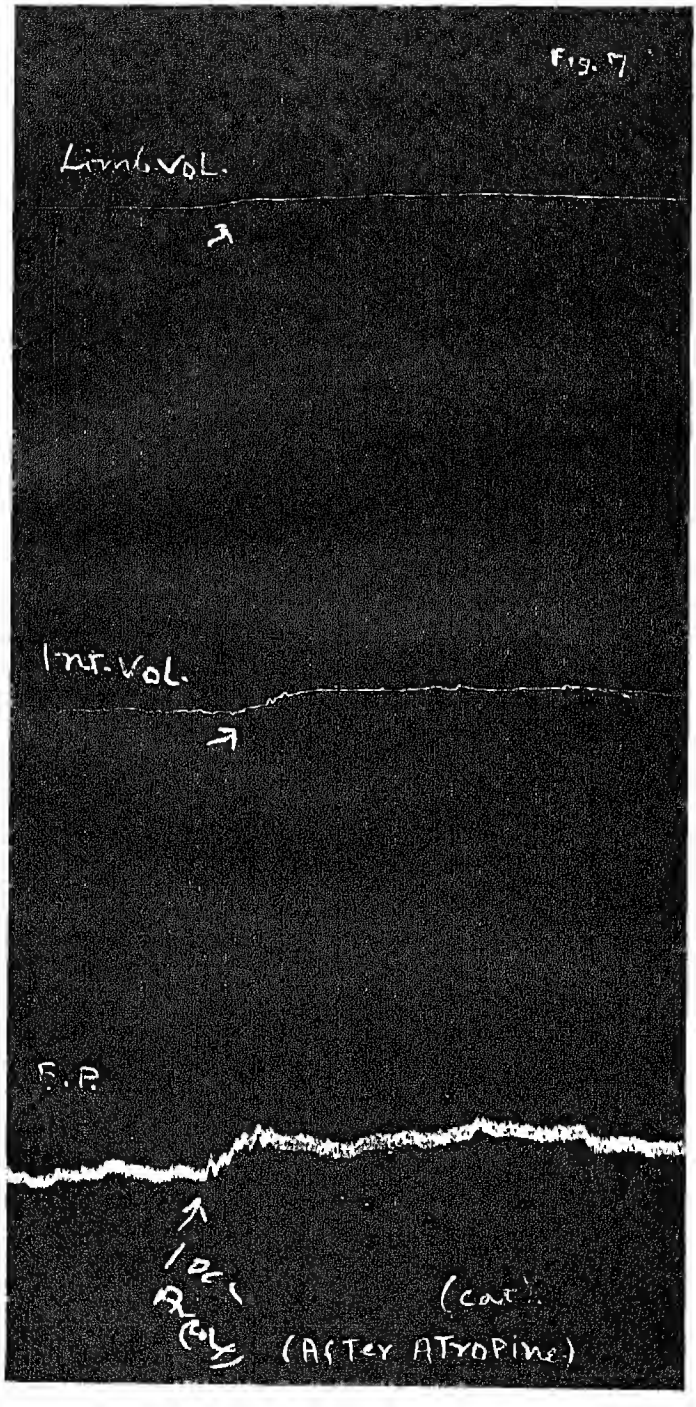


Fig. 7.—Injected at the point of arrow; note the increase in B.P. with slight changes in the intestinal and limb volumes.

PRELIMINARY OBSERVATIONS ON *TERMINALIA ARJUNA* W. & A.

by D. K. Roychoudhuri, N. K. Das Gupta, *and* B. N Ghosh, *Department of Pharmacology, R. G. Kar Medical College, Calcutta.*

(Communicated by B. Mukerji, F.N.I.).

Introduction

Arjuna is reputed to be a cardiac tonic in the indigenous materia medica for pretty long years. It is said that Vagbhatta was the first to prescribe the bark of '*arjuna*' in heart disease. Subsequently Chakradatta, the great Hindu physician described it as a cardiac tonic and astringent. The bark powder was used as a decoction with milk and treacle water or 'Ghreeta' i.e. preparation with ghee or melted butter. (Chopra R.N. 1933).

Habitat

Terminalia arjuna is a large deciduous tree of the lower Himalaya, Bengal, Burma, Central & Southern India and Ceylon. There are as many as 15, some say 20, different varieties of *Terminalia* which have much similarity in appearance and all are popularly known as '*arjuna*'. Caius, Mhaskar, Isaac (1930) made the following observations in this connection that as the pink coloured barks of *T. arjuna, Coriacea pallida,* etc, are commonly mistaken and sold indiscriminately as *arjuna,* scientific pharmacognosy is the only reliable method for determining their specific differences.

Chemical Composition

Identification of the proper species of *Terminalia* is very important, as the ash content and mineral constituents of the different varieties of the bark varied considerably. Caius, Mhaskar, Isaac (loc cit) however, could not find any alkaloid or glucosidal body in any of the different varieties of *Terminalia* examined by them Earlier Ghosal (1909) made a chemical and pharmacological study of the bark of *T. arjuna.* He found it to contain the following substances:—(1) sugar, (2) glucosidal body, (3) calcium carbonate, (4) Tannin etc Chopra and his associates (1929), however, were unable to find any active substance like alkaloid or glucoside On the contrary Agarwal & Dutt (1935 & 36) found a colourless crystalline compound which they called 'arjunin' $C_{26}H_{32}O_3$, m.p —192°C, a dark red amorphous colouring matter m.p.—132°C, 'arjunetin' $C_{11}H_{18}O_4$, H_2O, m.p —215°C and reducing sugars.

The present investigation was undertaken at the R. G Kar Medical College, pharmacology laboratory and the samples of the bark of *T. arjuna* were received through the courtesy of M/S Bengal Chemical & Pharmaceutical Works Ltd., Calcutta. The findings of the different workers on the chemical composition of the bark including that of the authors are tabulated for comparison (Table No. 1).

Pharmacological Activity

So far pharmacological activity has been reported to be either negative or discouraging except that by Ghosal (1909). He found it to be (a) a cardiac stimulant and tonic, increasing the force of contractions, slowing the rate but never completely stopping it. (b) a pressor substance increasing the blood pressure by constricting the peripheral arterioles probably by acting through the vasomotor centre. (c) a powerful haemostatic, only drawback being its pressor effect. (d) a weak lithontriptic, by increasing the excretion of phosphate and uric acid.

Caius *et al* (1930) however, found *T arjuna, belerica,* and *pallida* to act as diuretic without having any effect on the blood pressure or on the volume of the kidney. Whereas *T. chebula, myriocarpa, paniculata, coriacea pyrifolia, bialata* (a) raised blood pressure; (b) increased rate volume and systole of the heart, (c) increased limb volume and (d) caused vasoconstriction in the splanchnic area. The action was irrespective of the vagus control or central nervous system. Chopra and his associates (1929) on the contrary did not find any pharmacological activity in *T. arjuna* except that due to the calcium ion which is present in the bark in considerable amount.

Present investigation under C. S. I. R. scheme has revealed definite activity particularly with a fraction herein called Ar(0)4. Table No. 2 shows the result of preliminary screening work with different fractions from barks of T. *arjuna* that was undertaken to find out the most active fraction. And at the present moment attention was concentrated towards this fraction, Ar(0)4 and attempts were made to explore its pharmacological activity on the different systems of the body.

As the amount of active substance obtained from the crude bark is very small and the method of its preparation which is detailed below is time consuming, the progress on the pharmacological investigation is naturally incomplete.

Method of Preparation of Ar(0)4

Dried bark powder of *T. arjuna* was extracted with 90% of alcohol in a soxhlet extractor for 14-16 hrs The extract was freed from alcohol and dried on a water-bath. The powdered mass was shaken with distilled water for 2-3 hrs The water extract thus obtained after filtration was shaken repeatedly with ethyl ether. This ether-extract was slightly yellowish in colour. The ether extract was allowed to dry at room temperature and the final residue was dried in a vacuum desiccator. This dry powder, which was slight yellow in colour, has been named here as Ar(0)4.

Chemically Ar(0)4 is a mixture of different substances, mainly organic in nature, which is partially soluble in water and almost completely soluble in alcohol. This is free from calcium The solutions are light yellow in colour. The sample has been put to chromatographic analysis using Brockmann's alumina. By this method we have obtained two different fractions—(a) colourless to light green, (b) yellow. Both of these are pharmacologically active on frog's heart. Further work to identify the fractions chemically and their detail pharmacological study is under progress

Pharmacological Activity of Ar(0)4

On frog's heart in situ.—Pithed frog was taken. Canula was introduced into the portal vein and frog's saline was run in very slowly, one of the branch of the truncus artoriosus was cut open to allow the fluid to be pumped out by the ventricle The drug was generally injected through a rubber tubing at the base of the canula and records were taken on a moving drum. Sometimes, prior to the injection, the drug was poured directly on the surface of the ventricle. This generally gave poorer results as compared to the injections. A control dose of frog's saline equal to the volume of the drug injected was always given to note the change that may be produced by this extra amount of fluid alone.

In small doses Ar(0)4 stimulates the force of contractions, this increase in amplitude of contractions was associated with slight or no corresponding increase of rate generally Upto a certain limit on increasing the dose the increase in amplitude of contractions was maintained; when this limit was reached, there was no further rise and the heart appeared at times refractory to further injections. In some experiments repeated injections or with a larger

dose, the heart became slow and the rhythm was altered, (Fig. 2, (a), (b) & (c).) Occasionally there was a pause between a series of regular contractions; subsequently the pause lengthened and ventricle stopped beating for a short period due to lowering of auriculo-ventricular conductivity. This blocking, however, passed off gradually. In cases of overdosage or in continuous perfusion experiments the blocking effect was persistent (Fig. 3, (a) & (b).) This blocking is also independent of vagus control, as destruction of vago-sympathetic trunks were without any influence.

On isolated mammalian heart—Excised mammalian hearts of guinea-pig's or kitten's were perfused with Locke's solution through which oxygen was continuously bubbled. Some quantity of defibrinated whole blood was added to the perfusing fluid to ensure a more natural condition Temperature of the fluid was maintained between 36°—38°C. Apparatus used was that of Gunn's.

The effect of Ar(0)4 was more or less similar to that observed in frog's, i.e. increased ventricular systole. But generally speaking, the slowing and the blocking effects were more manifest than the initial increase in amplitude of the ventricular contractions (Figs. 4 & 5, (a), (b) & (c).)

Effect on Blood pressure (dogs and cats)

Generally speaking the effect on blood pressure is not marked In some instances here is a tendency towards fall specially in dogs which, however, soon disappears In some experiments there is a tendency towards rise which though not marked do not show any fall especially in cat. A definite rise occurs following section of both vagi or after administration of atropine. It is premature at this stage to say whether these changes in B P. are due to differences in the species or the samples used or whether due to central or peripheral effects.

Spleen, intestinal and limb volumes increased during the fall of blood pressure, slowly coming back to normal No marked change in the volumes, however, occurs during the period in which the blood pressure tended to rise after vagal section (Figs. 6 & 7).

Discussion

From the results so far obtained, *Terminalia arjuna* appears to be pharmacologically active. The effect was not due to calcium ion, since Ar(0)4 which used in the investigation was free from calcium. From the results of preliminary screening work, it appeared that the fraction herein called Ar(0)4 was the most potent one

Both on frog's and mammalian hearts smaller doses of Ar(0)4 cause increase in amplitude of contractions, somewhat resembling the effect produced by adrenaline (Fig 1, (a) & (b)) But it differs from adrenaline in two respects—(i) showing less effect on the rate of the heart; (ii) development of heart block with larger doses This presumably suggests that the effect of Ar(0)4 is more on the auriculo-ventricular node or the myocardium than on the myoneural junctions, as adrenaline in large doses produces ventricular fibrillation. On the other hand the combined stimulation and blocking effects, have some similarity to that of digitalis group of drugs. Ar(0)4 however, differs form digitalis in, (i) lack of latent period in action specially in frogs, (ii) increasing the rate instead of slowing in small doses In fact the blocking effect is more readily produced in mammalian hearts whereas in some frog's heart no blocking can be produced even with repeated larger doses.

TABLE No. 1.

Worker's name.	Total ash.	Inorganic elements.	Organic elements.
Hooper (1891). Quoted by Chopra R N., (1933)	34%	Calcium Carbonate.	Tannins—16%
Ghosal (1909).	30%	$CaCo_3$, $NaCo_3$, Traces of Chlorides of alkali metals.	(a) Sugar, (b) A Glucosidal body (c) Tannin—12%, (d), Colouring matter.
Chopra, R. N., (1929) & his associates	?	Calcium (large quantities). Traces of Almunium, Magnesium.	(a) Sugar, (b) An organic acid, with high melting point and a phytosterol, (c) An organic ester easily hydrolysed by mineral acids, (d) Tannin—12% (of pyrocatechol type), (e) Some colouring mater. N.B.—No alkaloid or glucoside.
Caius, Mhaskar & Isaac (1930).	26.78%	CaO —14.995% Co_2 —10.602% MgO — 0 280% P_2O_5 — 1.065% SO_3 — 0.119% Cl — 0.220% K_2O — 1.017% SiO_2 — 0.051%	(a) Tannin, (b) Colouring matter. N.B —No alkaloid, glucoside or essential oil.
Quoted by K L. Dey (1896).		$CaCo_3$—30.0%	Tannin—15%
Authors' findings.	13%	Calcium, Traces of strontium, iron, Magnesium, Almunium, Silica, Sulphate & Sodium.	(a) Sugar, (b) Glucosidal body, (c) Alkaloid, (?), (d) Tannin, (e) Colouring matter, (f) Gummy material.

TABLE No. 2.

Screening of Different fractions

Name of the fraction.	Effect on frog's heart.	Effect on mammalian heart.
Ar W. (watery extract)	Active (+)	Not tried.
Ar. P (Petroleum-ether extract residue)	Nil.	Nil.
Ar A (Stas-Otto-1st fraction)	Active (+)	Not tried.
Ar B (Stas-Otto-2nd fraction)	Doubtful (±)	Not tried.
Ar. C (Stas-Otto-3rd fraction)	Nil.	Not tried.
Ar 1 (Ethyl-ether extract residue)	Active (+ +)	Active (+ +)
Ar. 1(a) (Above slightly modified)	Active (+)	Not tried
Ar. 2 (Ethyl alcohol extract residue)	Active (+)	Not tried.
Ar(0)1 (Chloroform extract at neutral pH residue)	Nil	Not tried.
Ar(0)2 Same extract at acidic pH residue)	Nil.	Not tried.
Ar(0)3 (Same extract at alkaline pH residue)	Active (+)	Not tried.
Ar(0)4 (Alcohol extract residue re-extracted with ether at neutral pH residue)	Active (+ + +)	Active (+ + +)
Ar(0)5 (Same re-extracted at acidic pH residue)	Active (+)	Not tried.
Ar(0)6 (Same re-extracted at alkaline pH residue)	Nil.	Not tried.

Summary

(i) *Terminalia arjuna* is pharmacologically active; Ar(0)4 is the most potent of the different fractions obtained from '*Arjuna*'.

(ii) Chromatographically Ar(0)4 is separable into two distinct substances both of which are pharmacologically active on frog's heart.

(iii) On both frog's and mammalian hearts, small doses augment the systolic contractions; with larger doses or repeated injections, heart block is produced.

(iv) It has negligible vaso-pressor effect in intact animals, slight rise of pressure occurs following section of the vagus or administration of Atropine.

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BIOLOGICAL ASSAY OF PREPARATIONS OF *RAUWOLFIA SERPENTINA* BENTH. FOR THEIR HYPOPIETIC POTENCY

(A Preliminary Report)

by P. K Roy, G. K. Ray, *and* B. Mukerji, *F.N.I., Department of Pharmacology, School of Tropical Medicine, Calcutta.*

Introduction

As a result of a large volume of work done on the chemical constitution and pharmacological action of *Rauwolfia serpentina,* the plant has been recognised as a useful hypotensive drug The short clinical experience with the drug has justified the claims made in the laboratories and the plant has attained an important position in the medical armamentarium as a useful agent for relief of symptoms in hypertension. The ever-increasing demand for the drug for this purpose has apparently stimulated production, and supplies of its various preparations are now freely available from more than one manufacturing concern.

Unfortunately, there being as yet no fixed standard as to the quality of the preparations offered for medicinal use, a large proportion of these are often spurious and adulterated having inconsistant effects, or undesirable side-effects. In order, therefore, to keep an effective check on the quality of Rauwolfia preparations, it is necessary that all preparations of the plant should be assayed for their hypopietic potency so as to ensure uniformity of action.

Hitherto, Rauwolfia preparations have been assayed for their hypopietic potency by quantitative chemical estimation of their alkaloidal content (Schroff and Bhatia, 1941, Dutt, et al, 1947), presumably because the alkaloids alone have so long been believed to be responsible for the hypopiesis produced by Rauwolfia preparations. As, however, the alcoholic extract of the drug appears to produce a larger fall of the systemic blood-pressure than the purified total alkaloids present in a similar quantity of the alcoholic extract it is apparent that some other constituent or constituents in addition to the alkaloids is/are responsible for the larger hypopiesis produced by the extract The known methods of quantitative chemical estimation, however, afford means for estimation of those constituents only whose chemistry is definitely known. While, therefore, it may be possible to quantitatively estimate the alkaloids, it is not possible to assay the hypopietic potency by chemical methods. It seems, therefore, that the hypopietic potency of Rauwolfia preparations can only be assayed biologically. In fact, it has been said (Munch, 1937) that bio-assay is a necessity when the active constituents of a product are chemically unknown or when chemical assay does not give indications of its potency An attempt has, therefore, been made to evolve a convenient method of bio-assay of Rauwolfia preparations and a preliminary report of this work is being communicated here

Procedure

Following the well established principle of Biological standardisation, the method of comparison of activity of a sample with that of the standard has been employed for evolving a method of assay for Rauwolfia preparations.

An alcoholic extract, 'Standardised to contain 1% of the total alkaloids' has been prepared by the authors, from the dried root of an active specimen of *R. Serpentina* Benth, of Dehra Dun variety, to serve as the 'Standard' This extract, stored in the laboratory for the special purpose of this investigation, has been periodically tested, chemically and biologically, and also by clinical use in the attached hospital, and thus a uniform potency maintained throughout

the period of investigation. "Standard" preparations of any other strength required for the purpose of the investigation have been prepared directly from this stock preparation.

Cats, irrespective of their sex, have been used as the experimental animal throughout the course of the investigation, their weight varying from 1.5 to 2 5 kg. The carotid blood pressure of the animal was artificially raised by injection of a given amount of one of the known hypertensive drugs; because such a procedure enhances the hypopnetic effect of Rauwolfia and thus facilitates comparison and narrows the limits of error.

The choice of a particular hypertensive agent and its dosage was rather difficult to make. Several of them e g adrenalin, pituitrin, veritol and hypertensin were tried and ultimately reliance was placed on hypertensin. Hypertensin was obtained by mixing 'Renin' with fresh cat's blood which in turn was prepared in the laboratory according to the method of Grossman (1938)

An attempt was also made to use "Histamine" as the "Standard" in place of R. Extract as that may be conveniently used with precision by everybody concerned without depending on a "Standard" of a particular laboratory Later on Priscoline was also tried for a similar purpose as the mechanism of fall of blood pressure caused by priscoline is very much similar to that of Rauwolfia.

Results and Conclusion

The results reported here are deduced from about one hundred experiments The effect of various hypertensive and hypotensive agents was studied in the preliminary series of experiments. In graphs I-VIII the effect of adrenalin, ephedrine, pituitrin and hypertensin on blood pressure is shown. The suitability of these agents for producing experimental hypertension was studied by seeing the amount of fall of blood pressure produced by Rauwolfia preparations. Hypertensin and Pituitrin were considered suitable for further work. Various samples randomly picked up from the market were then compared with the standard preparation. In Graphs XXII to XXIV the result of such experiments is shown. Quite a few samples were found considerably weaker than the standard and this further stresses the need for bio-assay of Rauwolfia preparations

Graphs IX to XIII show the effect of crude alcoholic extract and alkaloids of Rauwolfia serpentina on blood pressure raised experimentally with Pituitrin In graphs XIV to XVIII the fall of blood pressure caused by Rauwolfia is compared with that of histamine in animals with blood pressure raised with pituitrin. It was found that 0 065 cc to 0.07 cc/kg of a 1 per cent alcoholic extract produces as much hypopiesis as 0.4 mg/kg of histamine.

Similar comparison between Rauwolfia and histamine was also carried out after hypertensin as a hypertensive agent and in this case 0.068 to 0 070 cc./kg. of 1 per cent Rauwolfia extract was found to be equivalent to 0.04 mg/kg. of histamine (graphs IXX-XXI) The margin of error in this case is narrowed down as compared with pituitrin as a hypertensive agent.

This work shows that histamine could possibly be employed as a standard for comparison with test samples of Rauwolfia and that Hypertensin is the most suitable hypertensive agent for this work. Further work to define the standard for Rauwolfia preparations and effect of storage on these is in progress.

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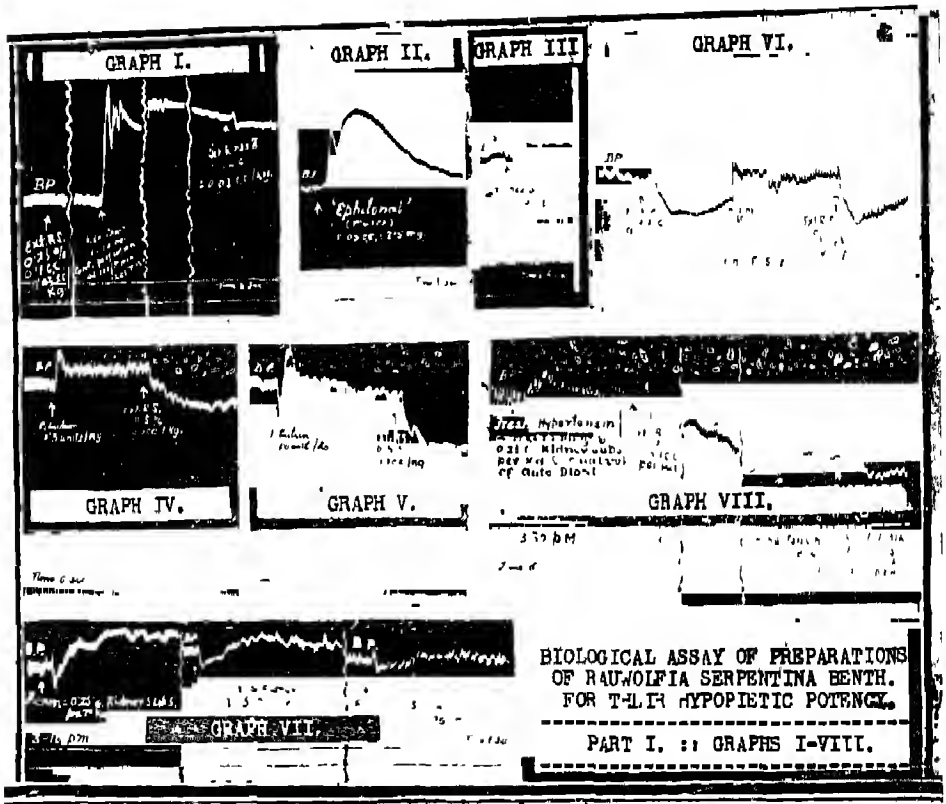
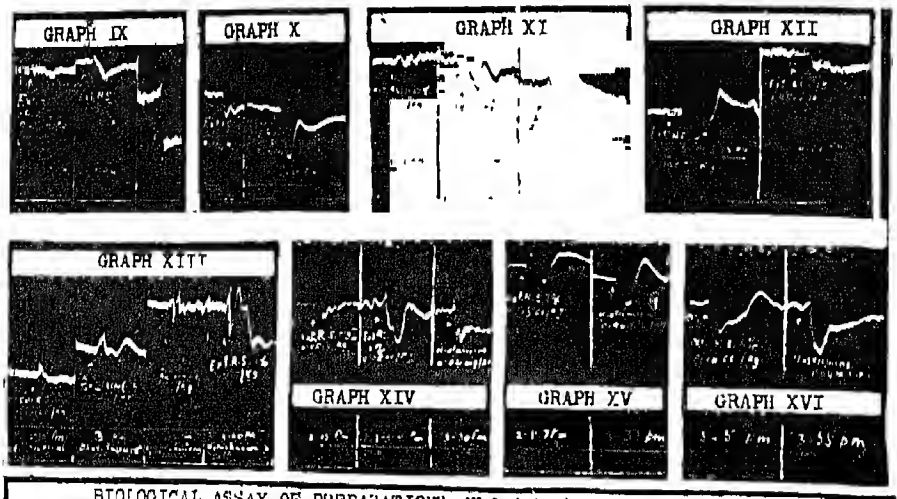
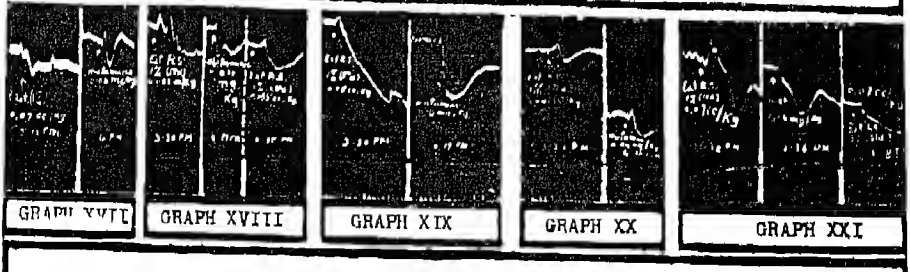


Interpretation of Graphs.

GRAPH I. ... Hypertension induced with Adrenaline & enhanced reaction to Rauwolfia after hypertension.
GRAPH II. .. Hypertension with Ephedrine; unsustained rise of B.P.
GRAPH III: . Hypertension with A.speciosa; small but sustained rise.
GRAPH IV: .. Hypertension with Pituitrin; 0.50 unit/kg.,i.v.
GRAPH V: ... Same with Pituitrin 1.0 U/kg.,i.v.; larger fall of B.P. with higher initial hypertension.
GRAPH VI: .. Same with Pituitrin 2.0 U/kg.,i.v.; larger fall of B.P. with higher dose of Rauwolfia.
GRAPH VII: . Hypertension with Renin (Kidney extract); gardual ineffectiveness of Renin with repetition.
GRAPH VIII:. Hypertension with Hypertensin; larger fall of BP. with the whole extract of Rauwolfia than with the total alkaloids only.



BIOLOGICAL ASSAY OF PREPARATIONS OF RAUWOLFIA SERPENTINA BENTH. FOR THEIR HYPOPIETIC POTENCY.
PART II . . GRAPHS IX-XXI



INTERPRETATION OF GRAPH.

GRAPHS IX & X---- Increasing fall of BP with increasing doses of the extract, alkaloidal concentration remaining constant.

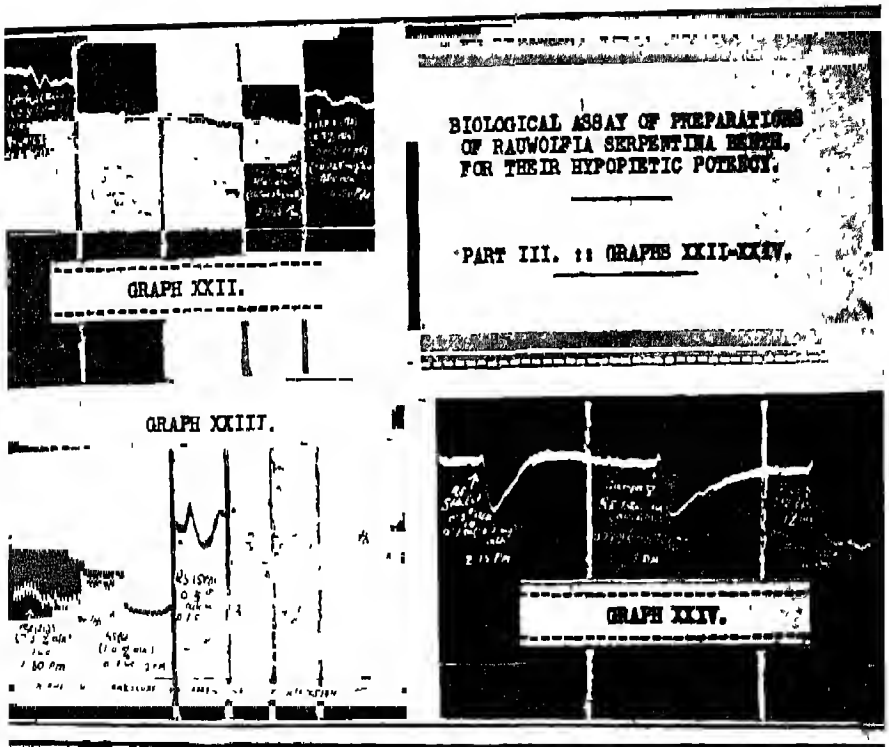
GRAPH XI - - - -: Increasing fall of BP with increasing alkaloidal concentration, dose remaining constant.

GRAPH XII - - -: Varying degrees of fall of BP with the same amount of total alkaloid in varying amounts of the extract.

GRAPH XIII - - -: Larger fall of BP with the whole extract than with the total alkaloids alone, both before and after hypertension.

GRAPHS XIV-XVIII : The fall of BP caused by R.serpentina measured in terms of the fall caused by a known amount of Histamine, after induced hypertension with Pituitrin.

GRAPHS XIX-XXI - : Same as above, after hypertension induced with freshly prepared Hypertensin.



Interpretation of Graphs.

GRAPH XXII.:- Test results of 3 sample preparations of RS.Benth., as compared with those of Laboratory Standards, after induced hypertension with Pituitrin.

GRAPH XXIII:- Test results of a sample preparation of RS.Benth. of unknown alkaloidal strength, as compared with those of Lab. Standards, after induced Adrenaline hypertension.

Incidentally, the graph also shows that the fall of BP. is enhanced after hypertension is induced.

GRAPH XXIV.:- Test results of a sample preparation of the total alkaloids of RS.Benth., as compared with those of Laboratory Standards, after induced hypertension with freshly prepared Hypertensin.

PHARMACOLOGICAL ACTIVITY OF RESINOUS RESIDUE OF *RAUWOLFIA SERPENTINA* BENTH.

by J. D. Kohli *and* B. Mukerji, *F.N.I., Central Drug Research Institute, Lucknow.*

Introduction

The hypnotic and hypotensive properties of *Rauwolfia serpentina* Benth. were first reported by Chopra, Gupta & Mukerji (1933). Several papers on the pharmacological action of the constituents of the plant have since been communicated. However, there have been contradictory reports regarding the hypnotic principle of the plant. Chopra *et al* (1943) concluded that the sedative and hypnotic properties are mainly present in the alcoholic extract as well as the total alkaloidal fraction from which ajmaline, serpentine and serpentinine had been removed. Gupta, Kahali and Dutta (1944) on the other hand found that the resin fraction washed free from all traces of alkaloids produced a sedative and hypnotic effect very similar to that produced by the standard extract of the plant. No other reference on the nature of activity of the resin fraction of the plant could be traced since 1944. The authors, therefore, undertook the investigation to verify the above findings of Gupta *et al* (loc. cit.).

While the work was in progress, Das Gupta *et al* (1953) recently reported that the oleoresinous fraction of *Rauwolfia serpentina* Benth. also exhibited a considerable sympatholytic activity while Gupta *et al* (loc. cit.) had reported that the resin did not cause a fall of blood pressure. According to Das Gupta *et al* (loc. cit.) this sympatholytic activity of oleoresinous fraction could be due to the weakly basic alkaloid *reserpin* isolated by Muller, Schlittler & Bein (1952), which might have escaped detection by the reagents which they employed for testing the presence of any alkaloid in their sample. However, this hypothesis could not be entertained in view of the work of H. J. Bein (1953) who reported that *reserpin* itself had no sympatholytic activity. On the other hand *reserpin* enhanced the action of sympathomimetic substances e.g., adrenaline, nor-adrenaline and ephedrine.

In view of this confused situation regarding the nature of the activity of the resinous fraction of *Rauwolfia serpentina* Benth., the scope of the present work was extended to include the study of general pharmacological action of resinous residue of the plant. The results of this study are being reported in the present communication.

Materials and Methods

Resin: The resin for the investigation was made available by the Chemistry Division of this Institute. The crude alcoholic extract of *Rauwolfia serpentina* Benth. (Dehradun variety) was concentrated *in vacuo* and the concentrate shaken out with chloroform. The chloroform soluble fraction was repeatedly washed with acidulated water and the residue obtained after removal of the solvent defatted with benzene. The benzene insoluble resinous residue thus obtained was tested for the presence of alkaloids with several reagents e.g.,

Mayer's, Dragendorff's and Picric acid reagents and consistently negative tests were obtained. However, nitrogen was found to be present when tested with Lassaigne's test. This fraction was insoluble in acids but soluble in alcohol An alcoholic solution of this fraction was used throughout this study. For purposes of comparison crude alcoholic extract and water soluble fraction of the alcoholic extract containing the alkaloidal salts were also employed in some experiments.

Hypnotic activity: Hypnotic activity of the resin was tested by antagonism to metrazol in albino rats and by objective observation in healthy rabbits

In rats, resin was injected intraperitoneally. The animals were challenged with metrazol 50 mg./kg. injected subcutaneously 3 to 4 hours after the administration of resin. All doses of the resin were contained in a fixed amount of alcohol (80%) which was 1 cc./kg. Alcohol and barbiturate controls were also put up side by side. The animals were observed for two hours after the challenge dose.

Rabbits were fed orally with alcoholic solution of the resin in the morning before feeding Food and water were allowed after giving the drug. Observations were started two hours after drugging and carried out for two hours at half an hour intervals. The animals were further observed morning and evening for two days.

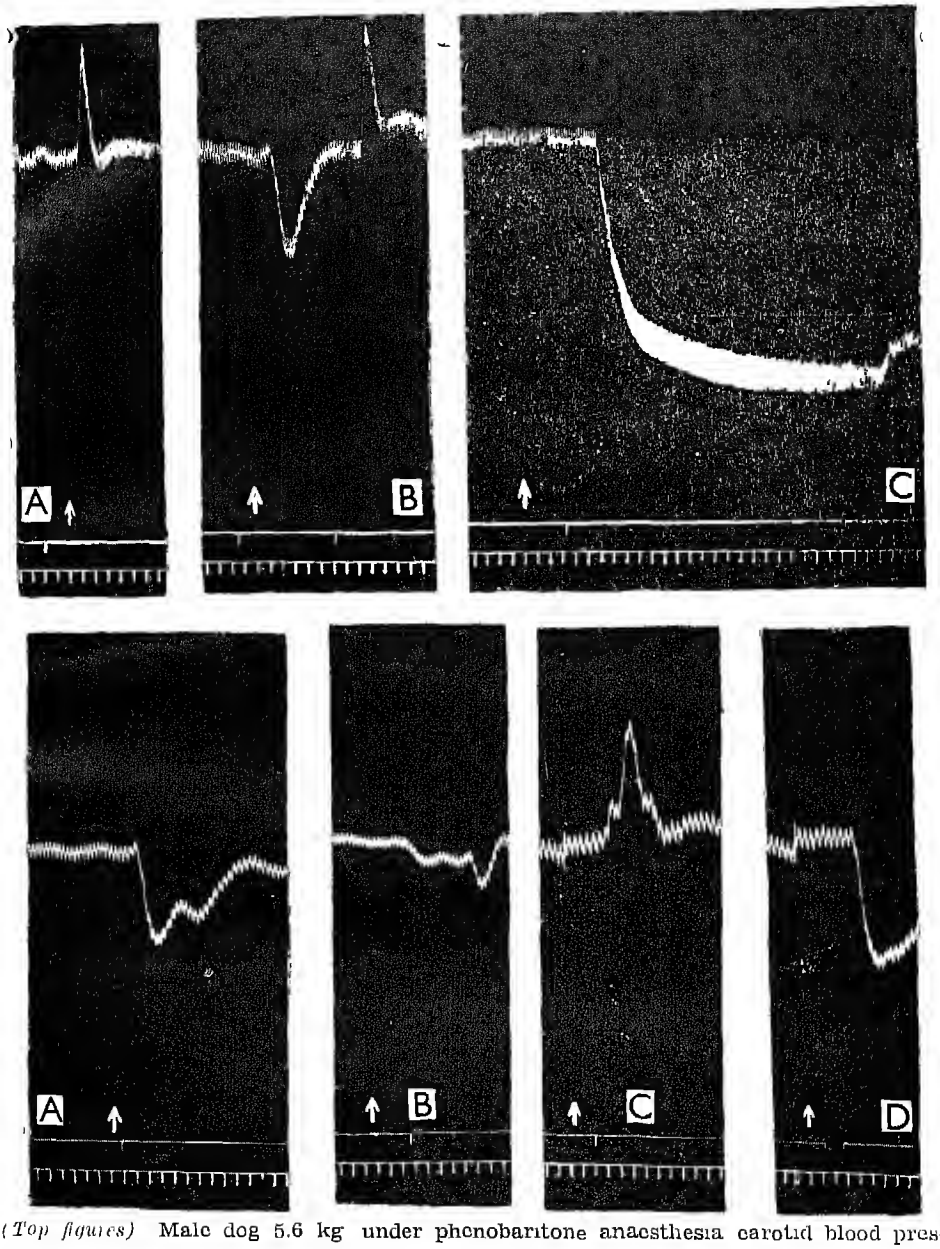
Pharmacodynamic activity: Pharmacodynamic activity of the resin was tested in dogs and cats under barbiturate anaesthesia with or without morphine Some experiments were done on spinal cats after ether anaesthesia. Carotid blood pressure was recorded with mercury manometer Respiration was recorded from tracheal cannula. Effect on intestinal contractions was measured by introducing a balloon in the small intestines. Effect on nictitating membrane of cats was recorded, following the technique described by Burn (1952).

The drugs were administered intravenously either quickly with a syringe or by slow perfusion from a burrette No saline was used to carry in the drug

Experimental Results

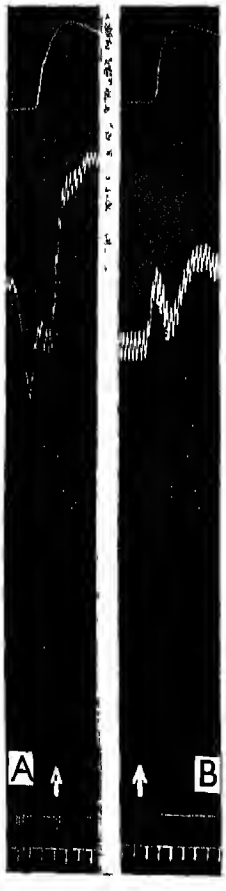
Hypnotic activity: 50 mg /kg. dose of metrazol caused convulsions in all the six unprotected rats. The severity of convulsions varied from jerky contractions of the body to the severe and repeated convulsions followed by prolonged tonic spasms of the body. This effect was more or less completely neutralised in 15 out of 15 animals by 25 mg./kg. of phenobarbitone sodium given intraperitoneally 3 to 4 hours before challenging. Ten animals were protected with 1 mg./kg. resin, 5 with 2 mg./kg. and 6 with 10 mg./kg. In all these animals convulsions appeared after metrazol. Similar results were obtained in 15 control animals injected with alcohol, 1 cc./kg., the same amount that was used as a carrier of the resin.

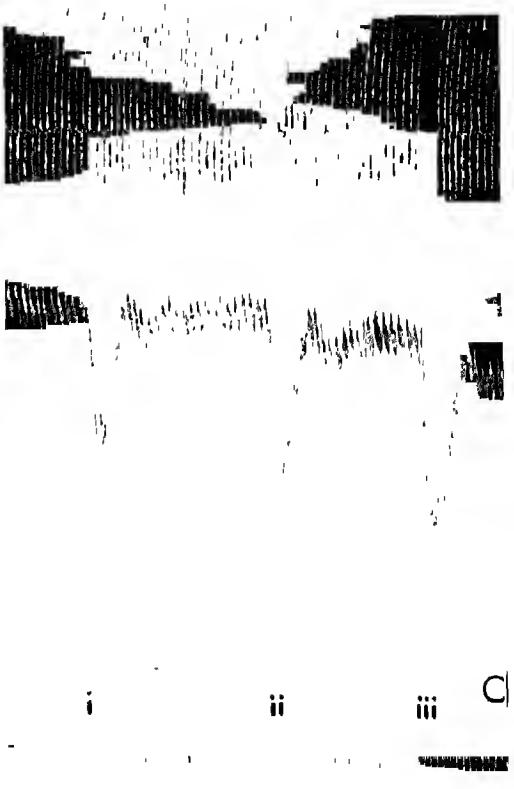
For feeding experiments rabbits were divided into 6 batches. Three batches acted as controls being fed water, alcohol and phenobarbitone sodium respectively. Among the other three batches, one was given resin 5 to 10 mg /kg. in alcohol which did not exceed 2 cc./kg Another batch was fed with crude alcoholic extract 2.5 cc /kg. The third batch was sub-divided into two groups (a) was fed 5 cc./kg. of water soluble fraction and (b) with 7 cc./kg. of the same stuff. (Five cc. of water soluble fraction could be considered to be equal to 2.5 cc. of alcoholic extract in the sense that each represented the same amount of the plant extractive). The results are tabulated below —



(Top figures) Male dog 5.6 kg. under phenobaritone anaesthesia carotid blood pressure.—Time interval 10 seconds.
A—10 micrograms of adrenaline
B—10 micrograms of adrenaline after 4 mg./kg. of resin
C—0.1 cc./kg. of crude alcoholic extract followed by adrenaline 10 micrograms.

(Bottom figures) Dog female 5.8 kg. under phenobarbitone anaesthesia carotid blood pressure.—Time interval 10 seconds
A—2 mg./kg. resin.
B—10 mg. acetyl choline
C—10 mg. acetyl choline after atropinisation
D—2 mg./kg. resin after atropinisation





Male cat 3 7 kg. under Barbiturate anaesthesia-upper record. nictitating membrane, lower record·- carotid blood pressure Time interval 10 seconds

A—Resin 0 5 mg /kg followed by 10 micrograms adrenaline during the fall of blood pressure.

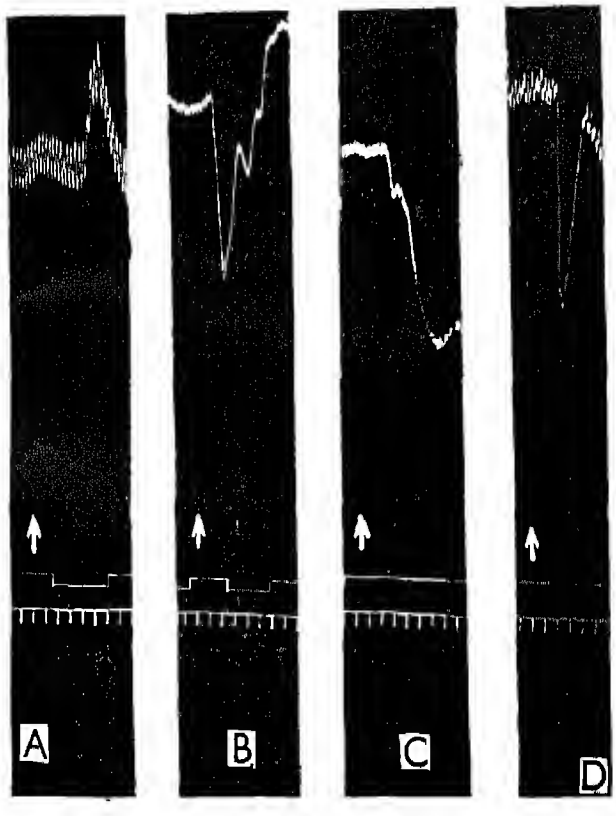
B —Adrenaline 5 micrograms.

C—Dog female 8.2 kg. under phenobarbitone anaesthesia; upper record: —respiration; lower record —carotid blood pressure

(i) 80 per cent alcohol 1 6 cc

(ii) Resin 2 mg /kg in 1 6 cc alcohol (80%).

(iii) Resin 4 mg /kg in 1 6 cc alcohol (80%).



Male cat 3.5 kg. under barbiturate anaesthesia, showing carotid blood pressure, time interval 10 seconds.
A—Adrenaline 5 micrograms.
B—Resin 0.5 mg./kg. followed by adrenaline 5 micrograms.
C—15 micrograms adrenaline given after Priscol, showing adrenaline reversal
D—Resin 0.5 mg./kg. after sympathetic block.

Substance.	Dose.	No. of animals.	Effect.
Water		12	No effect was noticed.
Alcohol 80% ..	2-2.5 cc./kg.	15	No effect was noticed with this dose of alcohol.
Phenobarbitone sodium	100 mg./kg.	19	Inco-ordination and dullness developed early followed by drowsiness. Animals, however, could be aroused.
Resin . ..	5 mg./kg.	4	No effect whatsoever could be noticed.
Resin	10 mg./kg	10	—Do—
Alcoholic extract .	2 5 cc /kg.	9	Dullness, drowsiness, contraction of the pupil and loss of righting reflex were noticed in almost all animals. The effects gradually decreased in intensity but continued for 2 days or so.
Water soluble fraction	5 cc./kg	11	Slight dullness was noticed in 3 out of 11 ammals.
Water soluble fraction	7 cc./kg.	11	Slight dullness was noticed in 6 out of 11 animals.

Effect on blood pressure: The resin in 2-3 mg./kg. doses in dogs and 0.5 mg./kg. in cats caused about 50% fall of the blood pressure. This occurs both in intact and spinal animals. The fall occurs immediately and complete recovery takes place in 5 to 7 minutes The time of recovery is much shorter than with crude alcoholic extract which is more than 30 minutes.

Injection of 5 to 10 micrograms of adrenaline at any time after the administration of resin produces a similar rise of blood pressure as before the resin This could be elucidated in several experiments (Plate XIV). In the same experiment 10 micrograms of adrenaline was injected during the hypotensive effect of crude alcoholic extract to show that the crude extract, in contrast to the resin, possessed adrenergic blocking activity.

In other experiments the animal was atropinized and evidence of peripheral blocking of para-sympathetic nerve endings was obtained by eliciting pressor response with Acetyl choline. An injection of resin at this stage caused the same amount of fall of blood pressure as before this procedure. (Plate XIV bottom figs.) In another set of experiments, sympathetic nerve endings were blocked by injecting Priscol or Ergotamine and evidence for the same was obtained by adrenaline reversal. Even under these conditions the resin caused a similar fall of blood pressure as before the block (Plate XVI). These experiments suggest that the hypotensive activity of the resin is not through autonomic mechanisms

Comparison with 80% Alcohol: A rapid intravenous injection of alcohol is known to cause a sharp drop in blood pressure (Sollman 1949). So before proceeding further to study the mechanism of hypotensive effect of the resin, effect of 80% alcohol which was used as a solvent for the resin was studied in

detail and compared with that of resin solution. Alcohol was given by slow perfusion at the rate of 0.5 cc per minute Respiration became shallow and slow gradually without any effect on blood pressure till near the end when respiration became markedly slow and blood pressure began to fall. The death occurred due to respiratory failure. Same effect was noticed when a solution of resin upto 5 mg./cc was given by slow perfusion. When artificial respiration was started before the heart stopped, the blood pressure returned to the original level, suggesting that the resin had no effect on circulatory system.

It could further be shown that the effect of alcohol or the resin solution depends on the speed of injection and there is little difference whether alcohol alone or a solution of the resin is given. In Plate XV-C the effect of equal quantities of alcohol, 80%, resin solutions, 2 mg./kg. and 4 mg./kg. in 80% alcohol is shown. It is clear that the extent of fall of blood pressure in every case is the same and depends upon the quantity of alcohol injected and not upon that of the resin. There is a momentary apnoea following injection which is perhaps the cause of fall of blood pressure.

Effect on other muscles: In several experiments put up to study the effect of resin on intestinal movement no effect of resin in doses employed could be seen. There was, also, no effect on the nictitating membrane in the above doses of the resin nor was the effect of adrenaline on the membrane altered in any way by the previous administration of resin. (Plate XVI-A,B,).

Conclusion

Experiments for testing hypnotic activity clearly show that this resin fraction produces no hypnotic or sedative effect in rats and rabbits in the doses upto 10 mg./kg. The crude alcoholic extract as well as the water soluble fraction, however, have marked hypnotic effect, which is of prolonged duration.

The resin solution causes a marked but transient fall of blood pressure both in intact and spinal animals. This effect of resin could not be adrenolytic, because a dose of adrenaline at any time after the administration of resin elicited the same response quantitatively as before giving the resin. Further that even after blocking the adrenergic nerve endings the resin produced the same effect as before. Nor was the effect of resin altered in any way in atropinised animals.

Later experiments where alcohol (80%) and the resin solution were given side by side and also the perfusion experiments show that the fall of blood pressure caused by resin solutions is due to the solvent and this fall varies according to the speed at which the solution is introduced into the system. The resin, therefore, has no effect on blood pressure. It also has no effect on other musculature under autonomic control.

From these experiments it may be concluded that this resinous residue of Rauwolfia serpentina Benth. is a physiologically inactive fraction in the doses employed.

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STUDIES ON THE ANTITUBERCULAR PROPERTIES OF *CUCURBITA PEPO* DC

by P. R. J. GANGADHARAM, *and* M. SIRSI, *Pharmacology Laboratory, Indian Institute of Science, Bangalore 3.*

(Communicated by B Mukerji, F.N.I.).

India has been the herbarium of the world from age-old times and our ancestors used to be the drug dealers with almost all the countries of the world Some of the drugs, indigenous to India, have been scientifically investigated, and are recognised by all the official Pharmacopoeias of the world. Nux-vomica, Senna, Opium are examples of such drugs enjoying such previleges. But there are many drugs whose usefulness is not fully realised through chemical and pharmacological investigations These drugs are only used to a limited extent, and that too by indigenous systems of medicine, whose traditional beliefs warrant a regular usage in clinical practice.

Centuries of successful clinical practice with many drugs in the native systems of medicine, should arouse our curiosity to investigate on a sound scientific basis their usefulness in combating illness. Many drugs of these systems of medicine are useful in combating some dangerous infectious diseases also, and it should be our sincere endeavour to place them on a firm scientific basis so that they can be used in combating these diseases.

One such drug, *Cucurbita pepo*, which is used in the Ayurveda system of medicine, as a specific for tuberculosis (Kirtikar and Basu 1918, Pandit) will be treated here *Cucurbita pepo* N. O. *Cucurbitaceae* (Synonyms: white melon, Ash pumpkin, *Boodigumbala, Kushmanda*) is cultivated in gardens throughout India and in many parts of the world. The fruit is used as a common household vegetable all over the country. Extract from the ripe fruit is regarded as a diuretic, tonic and is used in painful micturition, calcareous affections and general urinary disorders, besides its specific use in tuberculosis.

The Ayurveda system of medicine entertains this drug as a specific for tuberculosis, and a preparation, made from the pulp, known as "*Kushmanda Lehya*" is much used in the treatment of this disease and as a restorative in convalescence (Sivakumaraswamy) Except for some references about the chemical studies on the oil obtained from the seeds, and the analysis of the fruit as a vegetable, no reference is available in the literature about its anti-tubercular properties.

In the studies on the chemotherapy of tuberculosis, the authors undertook to investigate the antitubercular properties of the fruit. The results in the few experiments conducted so far will now be presented.

At the outset the "*in vitro*" studies with a crude [illegible] prepared from the fruit, for its [illegible] were [illegible] extract was prepared as follows:

The outer skin and the inner seeds having been removed, the fleshy part of a ripe, well-preserved fruit was minced with an equal quantity of water in a waring blender, the extract was concentrated to half its volume over a water-bath and strained through muslin. Further concentration was carried out under reduced pressure and finally dried over calcium chloride in a desiccator The yield was 10% of the original weight. A brown sweet-smelling syrupy liquid, of the consistency of treacle, of pH 6.8 was the product obtained.

The "*in vitro*" tuberculostatic activity (Sirsi, Gangadharam and De, 1951) was first determined in Youman's synthetic liquid media, using D_{37} (a

virulent strain locally isolated) and $H_{37}R_v$ strains of Myco. tuberculosis by the usual surface-culture methods (Sirsi 1951). The extract inhibited the growth of those virulent strains completely in 1/10,000 dilution and retarded more than 50% of the growth in a 1/100,000 dilution.

The tuberculostatic action was next tested by incorporating the various dilutions of the extract in a rich nutrient solid media (Petrick's (Gradwohl 1948) media gave the best results in our studies) and seeding varying amounts of different strains of *Myco. tuberculosis*. Tests were made in duplicate, the results being noted at the end of 3 weeks.

TABLE I

Antitubercular activity of a watery extract of *C. pepo* in Petrick's media.

Concentration of the extract.	Strain of Myco. tuberculosis.		
	D_{13}	$H_{37}R_v$	B.C.G.
1/100	—	—	—
1/1000	—	—	+
1/10,000	+	2+	2+

— No growth, + to 2+ various grades of growth.

Table I summerises the results obtained against an inocula of 0.1 mg. of tubercle bacilli. The growth of the fresh virulent strain D_{13} (isolated locally) was partially inhibited at 1/10,000 dilution. Complete inhibition of both the virulent strains was obtained in 1/1000 dilution while the action against the non-virulent B.C.G was of a much lower order.

The general antibacterial activity of the extract against some non-acid-fast organisms was determined by the standard turbidometric method, with the results shown in Table II.

TABLE II

Bacteriostatic Activity of *C. pepo*

Organisms.	Extract concentration.		
	1/100	1/1000	0
Staphylococcus aureus ..	—	+	+
Streptococcus pyogenes ..	—	+	+
Bact. coli . ..	±	+	+
Bact. typhosum ..	±	+	+

± Slight growth.

While the extract retarded the growth of a virulent strain of *Myco. tuberculosis* in 1/10,000 dilution, the bacteriostatic action against the gram positive

and gram negative bacteria tested above was very slight, indicating thereby that the extract like the p-amino salicylic acid, exerted a specific action against the virulent acid-fast *Myco. tuberculosis.*

Having determined the *"in vitro"* tuberculostatic action of the crude extract, evaluation of the *'in vivo'* activity of the extract in Experimental Murine Tuberculosis was undertaken. The method of testing was as follows.

Our laboratory strain of mice, whose susceptibility to tubercular infection and dose mortality curve had been previously established (Sirsi and De—), were infected intravenously with 1.0 mg. (wet weight) of $H_{37}R_v$ strain of tubercle bacilli The experimental animals were fed with 1 gm of the crude extract each by the drug-diet method. The mode of evaluation was as follows.

The gross amount of tuberculosis (macroscopic) was observed and recorded as follows (Sirsi and De—):

0-Apparently normal; 1^+ involvement of less than 10%, 2^+ involvement of 10-25%; 3^+ involvement of 25-50%; 4^+ involvement of more than 50% of the organ. The results are included in Table III

TABLE III

Effect of crude extract of *C. pepo* in experimental Mouse Tuberculosis.

Experiment	No. of mice.	Mortality.	Average survival time (days)	Average Wt loss or gain	Average amount of gross pulm. tuberculosis	Type of lesion
Controls ..	10	80%	24.0	—5 7	3.5	N.E.
Treated with *C. pepo* ..	10	70%	26.5	—4	3.0	P. & N.E

N.E.=Necrotic; P=Proliferative.

The results though not comparable to those of Streptomycin or p-amino salicylic acid (Youmans, 1949), are sufficiently suggestive to warrant further study. The slight increase in the survival time, the diminished gross involvement of the lungs, and the presence of more proliferative lesions indicate the chemotherapeutic efficacy of the drug, though only of a slight degree. Considering the crude nature of the drug, and the heavy intravenous dose of tubercle bacilli, attempts were made to isolate the active principles.

Various methods of extraction using different organic solvents were attempted, both from the fresh and dried pulp of the fruit, and all the fractions obtained from time to time were simultaneously tested for their *"in vitro"* tuberculostatic action. During the trials, extraction of the fresh juice or pulp or cold extraction of the dried pulp could not effect the isolation of the active principle, as is shown by the *"in vitro"* tests The hot continuous extraction with different solvents was, therefore, adopted to isolate the active substance. By the soxhlet extraction of the dried pulp with pure acetone, it was possible to isolate an active principle This substance named by the authors as "Peposin" is found to completely inhibit the growth of the virulent H_{37} H_v strain in Youman's media at a dilution of 1/50,000 for a period of 3 weeks, as determined by the surface culture method. The process of isolation in brief is as follows:

The fleshy part of the fruit, after separation from the seeds and the outer skin, but containing all the juice, was thoroughly minced in a blender, and then dried in a hot air drier (temperature not exceeding 40°C.) The dried pulp was then powdered and extracted with pure acetone (b.p.53-55°C) in a soxhlet continuous extraction apparatus for 40 hours A reddish-brown resinous mass settled at the bottom of the flask, while the supernatant acetone extract, was then decanted and concentrated under reduced pressure. On keeping in the frigidaire, a greyish white granular substance settled at the bottom of the flask. This was separated from the liquid by centrifugation at 4,000-5,000 r.p.m. and then dried over calcium chloride in a desiccator kept in cold storage.

This substance, as also the reddish-brown resinous mass were tested for their antitubercular action in Youman's media, with the $H_{37}R_v$ strain by the usual surface culture method. The results are presented in Table IV.

TABLE IV

Antitubercular activity of Peposin and the Resinous mass.

Substance.	Dilutions.					
	1/100	1/1000	1/10,000	1/50,000	1/100,000	Controls
Peposin . .	—	—	—	—	+	2 +
Resinous mass . .	+	+	+	+	+	2 +

— No growth; + to 2+ Various grades of growth.

Thus it is seen that "peposin" exerts considerable tuberculostatic properties. It is found to be soluble in chloroform, ether, and acetone, and moderately soluble in methanol, benzene, water and insoluble in alcohol and ethylene glycol. Further purification, stability, toxicity and the '*in vivo*' activity of this substance are under progress.

ACKNOWLEDGEMENTS

Before we conclude we must express our sincere thanks to Dr. K. P. Menon and Dr A S. Ramaswamy for their kind interest in the work.

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BIOCHEMISTRY OF PLANT PRODUCTS.

POSSIBILITY OF PREPARATION OF PEPTONES FROM OIL-SEED CAKES

by S. C AGARWALA *and* K. C. SAXENA, *Central Drug Research Institute, Lucknow.*

(Communicated by B. Mukerji, F N.I.).

Peptones are an essential constituent of various kinds of media used for growing micro-organisms (—1941,—1948). Ruff (1945-48) attempted to prepare meat peptones suitable for the production of Diphtheria toxin and came to the conclusion that samples containing amino nitrogen to the extent of 20-25% of the total nitrogen were the most suitable Topshtein (1940) attempted simular preparations of peptone for bacteriological purposes and suggested that those having high free amino nitrogen were the best. In contrast to the above workers, Hook and Fabian (1943) prepared peptones from vegetable sources like corn gluten and chemically analysed the samples. Decolourisation resulted in a loss of nutrients as shown by the decrease in growth and increase in the lag phase of *Escherichia coli.*

In India the entire requirement of peptones has to be imported because of the absence of any suitable local preparations It was, therefore, considered of importance to make an attempt to prepare peptones from indigenous sources like oil cakes, available as by-products from oil crushing industry Results obtained are described in this preliminary communication.

Oil cakes of ground-nut, sesame and mustard were obtained from the local market. They were ground to a fine powder in a milling machine and were continuously extracted with ether for about eight hours to make them free from oil. After drying, 500 gm of the extracted fat free powder was suspended in 3 liters of distilled water and thereafter 15 gm. of papain, finely dispersed in 100 ml. of water, was added. The pH of the fermenting broth was adjusted to 7.0. A few ml. of toluene was added and the digestion was allowed to proceed at 37°C

The course of hydrolysis was followed by the determination of free amino nitrogen in the filtrate by the formal titration at various intervals The results of a typical experiment are given in Table I.

TABLE I.

Increase in amino N_2 by digestion with papain.

No. of days of incubation.	Amino N_2 per 100 ml of broth
0	0.084 gm.
3	0.284 ,,
6	0.462 ,,
10	0.630 ,,

After ten days the broth was filtered and heated to coagulate the undigested proteins. The filtrate was then cooled and saturated with commercial

ammonium sulphate to precipitate the proteoses. Excess of sulphate was removed by boiling with barium carbonate. The final solution was concentrated under vacuum at about 60-70°C and dried over conc. sulphuric acid in a vacuum desiccator.

The yields obtained by this method were about 1.5 to 2% which are rather low. This yield may increase with the improvement in technique.

The sample thus obtained was compared with 'Difco' peptone as far as the growth promoting activity was concerned. Cultures of *Staphylococcus aureus*, *Escherichia coli* and *Vibrio cholerae* were grown in both the peptones and turbidity readings were taken in Lumetron photoelectric colorimeter with red filter (650 mμ). Uninoculated medium was adjusted to 100 and the results obtained are shown in Table II.

TABLE II.

Comparison of the growth of certain cultures in the peptone media.

Culture.	% Transmission. Difco.	Seed-cake peptone.
S. aureus	66	65
E. coli	63	64
V. cholerae (Ogawa 52)	84	84
Water Vibrio (Water tank)	80	76

It would be seen from the above results that as far as growth of micro-organisms is concerned, the peptone prepared from oil cakes compares favourably with the 'Difco' product, which is one of the best available. It may, however, be pointed out that the preparation reported above was highly coloured and hygroscopic. Decolourisation by charcoal resulted in a product which did not support the growth of organisms.

Further work is in progress to increase the yields and devise a suitable process for decolourising the product.

SUMMARY

Oil-seed cakes have been used to prepare peptone, the hydrolysis being carried on by papain. The product compares favourably as far as growth promotion is concerned but it is highly coloured and hygroscopic.

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SECTION D

THERAPEUTICS OF PLANT AND MINERAL PRODUCTS.

USE OF INDIGENOUS DRUGS IN PSYCHOLOGICAL MEDICINE

by M. V. GOVINDASWAMY, F.N.I., *Mysore Mental Hospital, Bangalore.*

Drugs are used in medicine for purposes of investigation, diagnosis and treatment. Their use might be specific, non-specific, or empirical. Hormone therapy and chemo-therapy are in general specific, while analgesics, anti-pyretics and others, used solely for the relief of symptoms, however great their value, are non-specific. Vitamins, antibiotics, anti-malarials, sulfa drugs, thyroid, and ovarian extracts are examples of specific drugs, while aspirin, and codeine, are of non-specific ones and the use of yohimbine and others are only empirical.

In psychological medicine, there can be no specificity of drugs, as understood in internal medicine, because there are no diseases, with clear cut etiology, pathology, and clinical course. The disorders met with in psychiatry, are individual types of reactions, with complex etiological factors, uncertain pathology, variable clinical course, and unpredictable prognosis Except in neurological illness, where mental abnormalities form only part of the clinical picture (e g neuro-syphilis, G P I, meningo-encephalitic syndromes in geriatric conditions, avitaminoses), in the majority of functional mental disorders, no structural alterations in the brain are to be found.

The brain is the sub-stratum of the mind and in psychiatry it is healthy as well as normal, with regard to histological, anatomical and physiological standards. Unless special techniques are developed to understand the subtle changes in cell units and homeo-static mechanisms, it will not be possible to appreciate what is happening in the brain and related structures in mental diseases. The radio-active isotopes, and the infra-red spectro-photometer has been of some help in this direction The Electro-encephalograph, has not so far fulfilled its expectations.

Another difficulty is that there is no method by means of which the activities of the brain could be correlated, or measured. Muscular activity can be correlated with thermal or electrical energy and glandular activity with the secretions produced or osmotic pressure generated. But there are no comparable methods for measuring the activities of the brain. Psychometry has not been found of much value for this purpose.

Psycho-analysis, might on superfical examination, be thought to be the specific treatment in mental diseases. But it is not so, because mental mechanisms are not so deceptively simple as depicted. Cultural and Sociological determinants of mental disorder are ill understood.

The recent advances in psycho-somatic medicine have also taught us that not only structure alters function, but function can also alter structure, in apparently unrelated systems like blood vessels, skin, gastro-intestinal and bronchial musculature, leading to anxiety states, high blood pressure, eczema, gastric and duodenal ulcers and asthma.

An appreciation of the multiplicity of the etiological factors in psychological medicine, is essential before a suitable treatment is formulated. The disorders encountered are individual types of reaction, comparable to syndromes in general medicine, that is, a group of signs and symptoms in a relatively constant configuration.

While specificity of treatment by drugs could be ruled out in psychiatry, the value of drugs, however, should not be underestimated. Drugs are used in psychological medicine, as sedatives, stimulants and anti-convulsants, to reduce psychological tension and resistance, for overcoming depressions, for shock therapy, for influencing the autonomic nervous system, muscular tone and electrolytic balance. Also for altering favourably homeostatic disequilibria.

Drugs now largely used are the barbiturates (luminal, sodium amytal) benzedrine, methedrine, sodium pentothal, evipan, paraldehyde, cannabis indica and its derivatives, cardizol and insulin for shock, concentrated vitamins (B_1, B_{12}, nicotinamide, ascorbic acid) protein hydrolysates, and amino-acids; plasma substitutes, bellergal, prisocophen and vago-lysin and others.

Most of the drugs mentioned above are expensive, complicated in their manufacturing techniques and have to be imported from America and Europe. A course of insulin shock for schizophrenic patients costs roughly two thousand rupees, including the cost of hospitalization. A valuable field for research would, therefore, be to investigate the possibility of getting from indigenous resources, suitable substitutes for the costly foreign drugs discussed above. Thus a drug may be found out which may not be anti-diabetic but effective in schizophrenic cases when used orally.

Epilepsy is taking an enormous toll in this country. At a conservative estimate, one in three hundred is epileptic, either overt, or potential—luminal, mesantoin, hydantoin group of drugs, mysoline are usually used in the treatment of epilepsy but all have their advantages and defects. The sociological implications of this disease are enormous It is an ancient disease, worthwhile investigating for an indigenous remedy with electro-encephalographic control.

The gonado-trope hormones are also to be taken into consideration. Vegetables like *Nugge Kai* (Drum-sticks) have acquired fame as sexual stimulants. The value of administration of puddings made of *'Till'* and *'Gur'* in South India to girls attaining puberty (*Chigale Unde*) has to be investigated scientifically. In the prevention of puerperal psychoses, does the administration of large amounts of ghee, and edible powders compounded with gum, pepper, sugar and various herbs play a part? These points are mentioned here, because functional diseases associated with puberty and puerperium in women are very much on the increase. So attempts may be made to search out drugs, of indigenous origin, with oestrogen like properties. Soya bean has been the subject of much intensive study in the preparation of synthetic oestrogens.

For the relief of endogenous depressions, in addition to electric convulsions, whose actions are not well understood, methedrine is being used with some effects but it has its disadvantages too. Some preparations of cannabis indica are likely to be equally effective This is a matter for investigation. Camphor and musk have to be investigated on similar grounds as stimulants Efforts should be made to prepare concentrated vitamins obtained from local indigenous sources, to prevent nutritional and geriatric psychoses.

Attempts may also be made to prepare from easily available groups of colloidal materials like resins, substances which would help to maintain osmotic pressure and electrolytic balances in dehydrated states, so common in mental disorder. Edible gums are found in plenty and they may be utilised for this purpose.

The importance of investigating drugs acting on the autonomic nervous system to reduce psycho-somatic tension, cannot be minimised. *Serpentina* is a pointer in this direction.

Carbon dioxide, sodium pentothal, and hyoscine derivatives are being used to reduce psychological resistance and facilitate psychotherapeutic procedures.

'*Datura*' is well known to produce mental abnormalities leading to unconsciousness and is being exploited by criminals. It is worth trying to prepare some drugs out of it, comparable in its properties to carbon dioxide, or pentothal sodium.

Drugs producing sudden reduction in temperature, without poisoning the nervous system, would help a great deal in inducing a type of hibernation, which would allay psychological anxiety. Attempts may be made to search out some such drugs from indigenous sources.

The value of cortisone is being deprecated now. Some doctors compare it only to aspirin. Bile salts (*Gorochane*) have been a valuable Ayurvedic remedy. Can its claims be maintained?

In neurology, the value of research to find out a suitable medicine from the indigenous sources can never be underestimated. While it is true that many neurological illnesses are progressive and degenerative in character, even if temporary relief of symptoms could be obtained, it would be a great boon There is also ample scope for intense investigations into the activities of indigenous drugs in reducing skin disorders, including leucoderma, asthma, mucus colitis, which are largely psycho-somatic in origin

It is one of the glories of Ayurveda to emphasise the integral relationship between body and mind, and the beginnings of modern psycho-somatic medicine could be traced to our ancient medicinal systems

In the present article, some of the problems relating to psychological medicine have been very briefly discussed and it is worthwhile for the various Drug Research Institutes and similar organisations to take up the work and give us the proper guidance in the line.

DIAMOND ON CANCER

by ANTUBHAI VAIDYA, *Director, Ayurveda Research Institute, Kalam Kutir, Frere Road, Fort, Bombay.*

(Communicated by B. Mukerji, F.N.I.).

So wonderful are the processes of growth governed by nature, that the more we probe into the physiology and pathology of it the more are we drifted into the darkness. The unit of creation of the human being is a fertilised ovum, which starts to develop and to suffer the laws of nature and in the end to die of the natural death according to one's fate. In the wake of post-natal life we study the human being and we find no apparant abnormality, we wait to observe and see what qualities are acquired unconsciously beneficial or prejudicial to one's existence, but we cannot foretell till the disease has made its appearance and we become helpless after it has made its appearance. We hit in the dark and try to explore the mystery of nature when we cannot arrive at any satisfactory and acceptable data in a particular being we think of the sperm and the ovum and start speculation over the genetics

So disappointing is the situation that we have not been able to find the cause of cancer. Pages of literature are written and will be written, but all speculations over the causative probability, and not the certainty. Cancer is the disease which humanity has suffered since times immemorial

Ayurved has its origin in the days of Atreya till 2600 B.C. If we are to take that as the probably oldest record of science then we find cancer being studied by the ancient Hindus in a perfect way; with no available means at their command they perfectly utilised their senses of touch, smell, sight and hearing I feel that nothing can compete nature in any harmonious perfection of the machinery. They concentrated all their senses over it, after observing the nature of the tumour and making a perfect study of the symptomatology. They tried to understand the nature of the underlying thing. We find classical description of the types of tumours in Ayurved. Sushuruta (whose period is still undecided matter), described nine main types of tumours malignant and benign.

He first described its classical character till it is fatal Then accordingly the morphology and gross appearances, and the subsequent changes it undergoes during the progress of the diseases, he classified them accordingly. Sarcoma, by its fleshy appearance he called it *Mansarbud*.

Then comes the period of darkness which prevailed during which the diseases were supposed to be due to God being displeased and the devil used to come to punish the individual in the form of disease for disobeying the God. There is no light thrown on the subject during the period which I call the dark period.

Hurriedly shifting ourselves to the modern age; it has been described as a mass of cells, or tissue, resembling that present in the normal body, but growing at the expense of the organism, without, at the same time subserving any useful purpose therein. The most striking thing about tumours is its complete autonomy over the body particularly, so in malignant tumours it takes its nourishment without any consideration to the state of nourishment of the host, for however the host be impoverished the tumour continues to flourish. Even now the nature of origin of tumour is a matter of absolute uncertainty, whether it starts from one cell or has the multicentric origin is still in the dark, but majority of tumours produce the local signs. So we may think that the origin is localised The principle on which the modern treatment of tumour in sur-

gery is based is the presumption that tumour to start with is a local process in majority of the types. With this much introduction the tumour may be defined as a mass of cells or tissue, in a particular organ rebelling the physiological laws of growth going out of way on the hostile path, just to continue to grow without subserving the useful purpose.

This mass may be simple or malignant, according to whether they prove fatal or not. Still the line of demarkation is not easy to determine because growth may remain relatively harmless for number of years, only to undergo a malignant change with the passage of time; so the distinction between the simple and malignant is not always possible.

Treatment

It is a very curious observation which I am humbly presenting today before the learned members of scientific world. It is our infeeling that this may prove to be very beneficial to the suffering humanity, empirically we have found the drug acting on malignant cells, which, if accepted by fair trials, will substantially contribute to solve one of the most important problems of the medical science.

The diamond has been found to be acting on the cancer cells of any origin. It is difficult to give the statistical data and the generalisation about its action on the malignant cell of any origin because of the limited scope for trial, cost of drug, etc.

The particular variety of diamond is subjected to the bio-chemical process and after having undergone that process diamond assumes the specific quality by virtue of which it ventures to fight against the human enemy.

The drug is given by mouth and starts acting evidently after eight days of administration. The dosages may vary in individual patients according to the stage of disease but very roughly it may be said that if at all the drug has to be effective it should be administered for at least a month and half to two months. The usual observation is that after the sufficient amount of drug has gone into the system in divided dosage; the patients starts reporting about amelioration of the symptoms, the pain gradually disappears and gradually enough the evident growth starts reducing in the size.

This drug was originally tried on a diabetic patient who had incidentally carcinoma tonsils with secondaries in the neck. It was supposed to be experimental for relief or cure of diabetes mellitus but very curiously I observed the secondaries in the neck reducing in size. This gave me the insight to work on the patients of cancer.

To cite the most recent example. I may put before you one case undergone my treatment.

> One female patient named Mrs T. V, age about 42 years, of moderate habits complaining of pain in the hip and sacro-iliac region with a non-fluctuating hard, tender, not red, not hot swelling, she was being treated by other doctors for about a year or more without any benefit. The plate advised by the previous medical attendant showed the bony growth of the right hip bone. The X-ray diagnosis was Myeloma giant-cell tumour

The X-ray report of Dr. A., Bombay.

> "Mrs T. V.: Ref. Dr. 2/m—
>
> *Conclusion:* The findings are definitely indicative of a bone neoplasm, most probably a benign giant-cell tumour although other tumours such as a reticulum cell sarcoma cannot be excluded without a biopsy."

Biopsy was taken by Tata Memorial Hospital on 10-10-1952. The report reads as follows:—

"The case No. M. 3903, Mrs. T. V.

Dear Dr. 2/m—

The aspiration biopsy of the lesion in the right thigh bone shows a plasma cell myeloma. She must be started on X-ray therapy.

Sd./—*Dr. 2/m*".

The same case came to me on 14-10-1952 with the above X-ray diagnosis and biopsy report. After about a year's suffering, the patient gave the history of pain in right hip region, inability to walk and bear weight on the affected limb. Onset was gradual. Duration, one year.

On examination I found clinically the swelling described as above; no history of blood in sputum, no history of cough.

The patient was put on the treatment of diamond. After six days of treatment, it was reported that she had considerable relief from the pain. After having taken the drug for 22 days clinically the improvement was noted.

There is no swelling visible; the patient walks without pain (though she is advised complete rest). She can bear the weight on that limb without pain. X-ray was repeated during the course of the treatment on 12-12-1952 by Dr M. whose report reads as follows:—

"Name of the patient: Mrs. T. V

Ref: Dr. 2/m—

Conclusion: Heart shadow is within normal limit. Lungs are clear. The above appearance suggests fibrocystic replacement of normal bone structure in the affected region Its nature appears to be of benign origin; not typical of any particular bone disease."

A biopsy was taken on 27-12-1952 by Dr S (Bombay) and his report reads as follows —

"Name. Mrs. T V.

Reference: Dr. 2/m—

Lab. Ref: No. 459.

Report on Examination of Biopsy material.

MICROSCOPIC EXAMINATION.

1. No organised tissue seen in the biopsy material.
2. No tumour cells seen.
3. No evidence of giant-cell tumour

REMARKS:—

Advised a careful rebiopsy.

Sd./- DR. 2/m—"

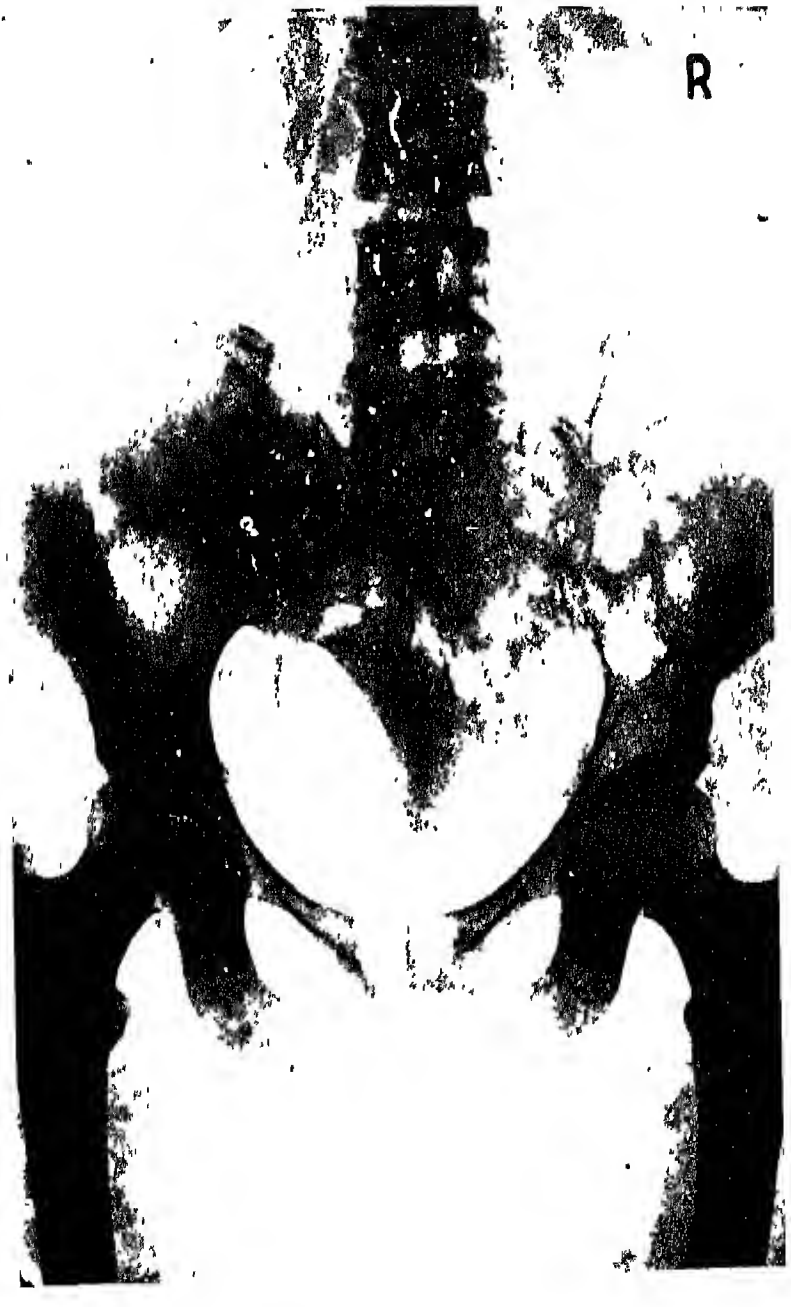
We still do not know how the drug acts; whether it reverts the malignant process or destroys the malignant cells and stimulates the normal process is not yet worked out. It is quite fair to speculate that the atoms of carbon in diamond after undergoing the special process might be undergoing such an activated change that they develop the selective tendency to act on malignant cells. This is only the possible explanation which can be given at present

Still there is tremendous amount of work to be done on the subject which may be summarized as follows:—

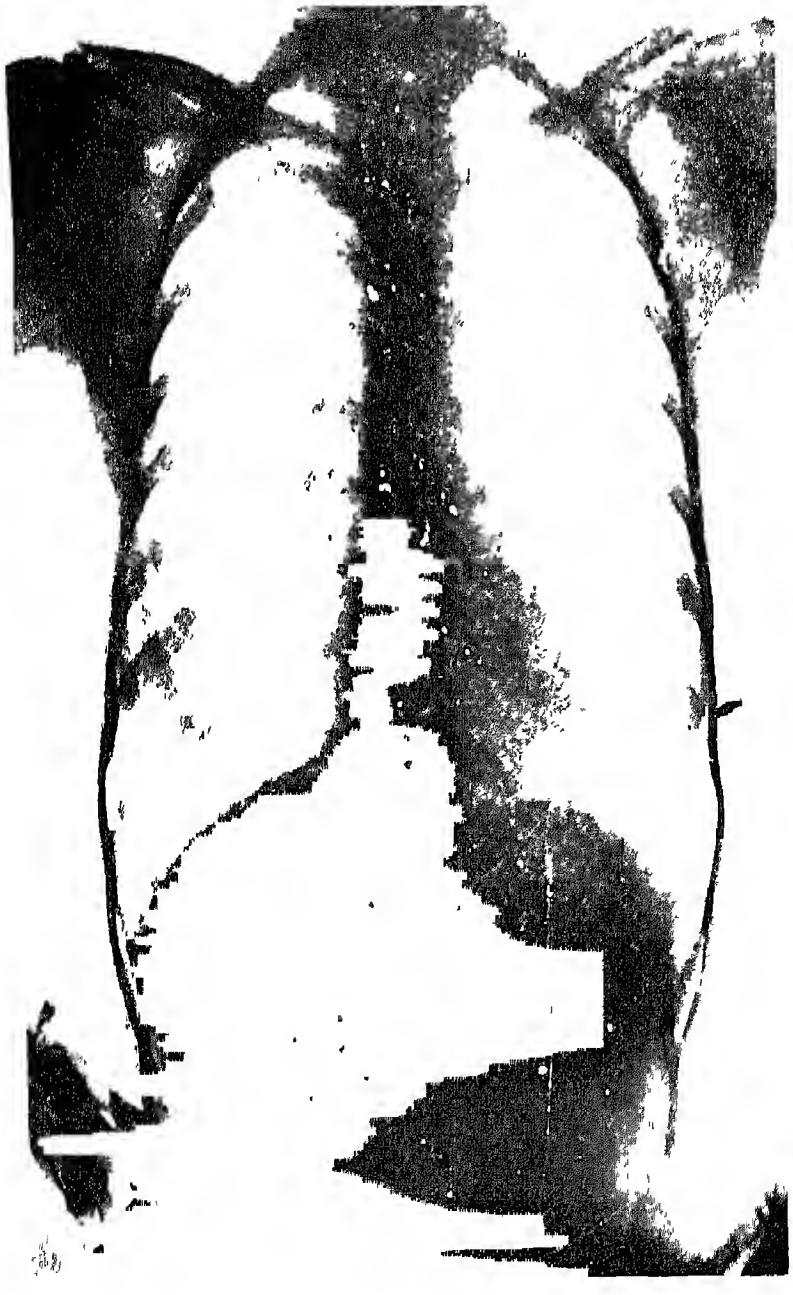
(1) To decide the method of action of drug.
(2) To find out the range of utility of drugs on cancer of various types.
(3) To find the drug resistant cases.
(4) To find the optimum stage when drug will have its maximum action.

X-RAY: SACRO-ILIAC REGION
of
MRS. T. V. (4-10-1952)

X Ray Sacro-iliac Region
of
Mrs. T V (10-10-1952)



X-RAY. SACRO ILIAC REGION
of
MRS T V. (12-12-1952)



X-Ray Chest
of
Mrs. T. V. (4-10-1952)

The limitations of finances, lack of proper co-operation from the authorities and the *non-prejudicial scientists* are the serious handicaps in the arriving at the proper data.

The bio-chemical characteristics are mentioned previously. The statement that the tumour cells differ in character and disposition of chromosome constituents of genus is still the fascinating ground to work on if we interpret the action of the drug in view of the biological character of the tumour cells we feel lost.

Formulae

The compound formulae finally adopted to treat the cancerous lesions in the human body is as follows:—

(a) *Drugs used by oral route.*

1. The powder (*Bhasma*) of the cortical crystals of PURE DIAMONDS.
2. *Tribulus terrestris,* Linn
3. *Crataeva religiosa,* Forst
4. *Bauhinia variegata,* Linn.
5. Asphalt.
6. *Hyoscyamus niger,* Linn.
7. *Cuscuta reflexa,* Roxb.
8. *Boerhaavia diffusa,* Linn.

(b) *Drugs used for local application.*

The paste of the drugs is applied in any type of cancer-affection.

(*i*) The powder of pure *Semecarpus anacardium,* Linn is mixed with lime water so as to form a paste-like compound

Prophylactic Treatment

The patient should not be exposed to cold. Perfect rest in the bed is absolutely necessary even in the early stages of invasion of this disease Exercise of any kind should also be entirely avoided.

The food of the patient must contain non-acidic materials.

The above mentioned treatment is proved successful in the 90 per cent of cases of cancer of breast and cervix.

In the end it may be concluded that the diamond subjected to the special bio-chemical process does act selectively on the malignant cells of probably any origin, the mode of action is presumed to be the selective affinity of the activated diamond towards the tumour cells; may be it might be preventing the further growth along with the visible retrogression in the existing growth, but most important observation is that it has no *prejudicial action on the normal cells,* and is not found harmful to the other vital organs.

Summary

The Investigation of "Role of Diamond on Cancer" was first taken up in the laboratory of Ayurveda Research Institute, Bombay in the year 1945. 326 cases of various type of cancerous lesions were put under observation. The result was remarkably successful, that is, about 60 per cent cases behaved normally by the administration of the diamond with the groups of its catalytic agents.

A recent case of Myeloma of sacro-iliac joint is put before the scientists for perusal.

SECTION E.

PHARMACEUTICAL BOTANY AND PHARMACOGNOSY OF MEDICINAL PLANTS.

EFFECT OF ROOT DIAMETER ON THE VARIATION OF ALKALOID CONTENTS OF *RAUWOLFIA* AND *DATURA*

by S. C. DATTA, *Central Drugs Laboratory, Calcutta.*

(Communicated by B. Mukerji, F.N.I.)

The gradation of roots according to different diameters is a common practice of the drug market and there is a variability of opinion regarding the presence of maximum quantity of alkaloids in thicker or thinner roots. A number of workers investigated Derris roots on this point but the results obtained by different groups of workers appear extremely divergent Thus, according to Moreau (1947) and Sievers (1940) the larger roots are inferior in alkaloid contents whereas Worsley (1934-35) reports the presence of a higher percentage of alkaloids in coarse roots as compared with those in thin roots According to Koolhaas (1938), however, the percentage of rotenone in extremely fine roots is however more than double of the amount present in coarser roots. Another group of workers again believe that roots of medium diameter contain the maximum quantities of the active principles and this view is supported by Maas (1935), Moore (1947) and Pagan and Hageman (1949).

Our investigation was confined to the roots of two plants, viz., *Rauwolfia serpentina* L. and *Datura metel* L. and it was observed that the roots of thinner diameters possessed a greater percentage of total alkaloids than the thicker ones.

EXPERIMENTAL

The roots of *Rauwolfia serpentina* were obtained from plants grown in the suburbs of Calcutta under natural conditions as well as from dried plants sold in the local market. In collecting the roots from plants, they were excavated as completely as possible and fresh roots were divided into the following diameter groups, viz., (1) Diameter up to 4 mm.; (2) Above 4 but up to 10 mm ; and (3) over 10 mm. After collection, all the roots were dried in the sun and the thicker roots were split to hasten drying. In case of dried roots obtained from the market, they were divided into different diameter groups as stated above, and the roots of each diameter group were powdered separately and passed through a No. 60 sieve The roots of *Datura metel* were obtained from cultivated plants grown on experimental plots for three successive generations from seeds supplied by Indian Botanic Gardens, Shibpore, Calcutta. The roots were collected in the same way as Rauwolfia roots and they were divided into the following diameter groups, viz., (1) Diameter less than 5 mm., (2) Above 5 mm. but less than 10 mm.; (3) Above 10 mm. but less than 20 mm. and (4) Above 20 mm. They were then dried and powdered as in the case of Rauwolfia roots.

The total alkaloids were estimated in the case of *Rauwolfia* roots by the method prescribed in the Pharmacopoeial List (1946) and is described below:

10 grammes of the powdered root were taken in a 500 c.c. stoppered flask and 200 c.c. of a mixture of 23 volumes of ether, 8 volumes of chloroform and 2.5 volumes of alcohol (90%) were added. The flask was then stoppered and shaken well and then allowed to stand for 10 minutes. It was then shaken fre-

quently with the addition of 6 c.c of dilute solution of ammonia. It was then allowed to stand for about 8 hours with occasional shaking. 10 c c of water were then added, the mixture shaken vigorously and when the powdered drug had settled, 100 c.c. of the solution representing 5 gms. of the drug were drawn out. It was then filtered through filter paper into a separator and washed with a few c c of a mixture of ether and chloroform. This extract was next shaken with successive portions of 20, 15, 10, 10, and 10 c.c. of N/2 sulphuric acid for complete extraction of the alkaloids. The combined acid extracts were collected in a separator and filtered through a filter paper wetted with water, washing the container and the filter with a few c.c. of water The total acid solution was then alkalined to litmus with dilute solution of ammonia and 5 c c. more of dilute solution of ammonia was further added The liberated alkaloids were then extracted with 20, 15, 10, 10 and 10 c c of chloroform and the combined chloroform extracts were washed with 10 c c. of water The water used for washing was shaken with two 5 c c. portions of chloroform and this chloroform washing was added to the main chloroform extract and the whole filtered into a tared 100 c.c. conical flask through a filter paper wetted with chloroform. The container and the filter were washed with 5 c.c. of chloroform and the chloroform was then distilled off on a water bath until only a few c.c. were left The solvent was then completely removed in a vacuum dessicator, 5 c.c of alcohol (90%) was added to the residue and the solvent was again removed. The evaporation with alcohol was repeated and the residue dried to constant weight in a vacuum dessicator and weighed as total alkaloids.

In case of Datura roots the assay was done according to the method prescribed under "Belladonna Herba" in the British Pharmacopoeia (1948) The percentage of alkaloids were determined in terms of 'hyoscyamine' and the details of the process are given below:

10 gms. of powdered roots were taken in a flask and 50 c.c. of ether-alcohol mixture (4·1) were added and shaken for 10 minutes, after which 1.5 c.c. of dilute solution of ammonia and 2 c.c. of water were added and shaken for one hour The mixture was then transferred to a percolator and the complete extraction of the alkaloid was affected first with 25 c.c. of ether-alcohol mixture and then ether. To the percolate 20 c.c. of N/2 hydrochloric acid were added and shaken well and allowed to separate and collected. The ethereal mixture was then extracted with further successive quantities of a mixture of N/10 HCL and alcohol (3:1) until the complete extraction of alkaloid was affected The mixed acid solution was washed with 10 c c of chloroform and the chloroform layer was run off in a second separator containing 20 c c. of N/10 HCL, shaken allowed to separate and the chloroform was rejected. This was repeated further with two quantities of 5 c.c of chloroform, washing the same acid solution as before. The acid solution from the second separator was transferred into the first separator and made distinctly alkaline with dilute solution of ammonium hydroxide. The alkaloid was completely extracted with successive quantities of chloroform. The combined chloroform extract was washed with 3 c.c. of water. The chloroform was transferred in a shallow open dish, evaporated and then 2 c c. of dehydrated alcohol were added to the residue and dried at 100° C. The residue was dissolved in 20 c.c. of N/50 sulphuric acid and titrated with N/50 sodium hydroxide using methyl red as indicator. The percentage of the alkaloid was calculated from the factor that each c.c of N/50 sulphuric acid is equivalent to 0.005787 gms. of hyoscyamine.

Results

The percentage of alkaloids obtained according to different estimations are given in the following tables.

TABLE I.

Percentage of total alkaloids in Rauwolfia roots.

Nature of roots	Alkaloid % according to different estimations.				Average % of alkaloids.
	(1)	(2)	(3)	(4)	
Thin roots (up to 4 mm.)	1.453	1.562	1 584	1.612	1.45—1 61
Thick roots (Above 4 mm. but up to 10 mm.)	1 216	1.224	1 234	1 226	1.21—1.23
Very thick roots (above 10 mm)	1 143	1.056	1.002	1.086	1.00—1.14

TABLE II

Alkaloidal percentage in Datura roots in terms of hyoscyamine

Nature of roots Diameter.	Alkaloid % according to different estimations			Average % of alkaloids
	(1)	(2)	(3)	
Less than 5 mm	0.278	0.275	0 277	0.275—0.278
Above 5 mm. but less than 10 mm	0.148	0.145	0 146	0.145—0.148
Above 10 mm. but less than 20 mm.	0.140	0.142	0.143	0.14—0.143
Above 20 mm.	0.135	0.138	0.134	0.134—0.138

OBSERVATIONS

It appears from the results of analysis stated above that in both the cases, the roots of thinner diameter possess a greater amount of total alkaloids. Microscopical examination of *Rauwolfia* roots reveals that the thinner ones possess greater number of oleo-resin cavities than the thicker ones Considerable quantities of starch are also found in the thicker roots and a sufficient number of oleo-resin cavities are found to be distorted and marked with degenerative changes. Microchemical reactions show the presence of alkaloids in the oleo-resin cells and this may explain the reason for the presence of a greater percentage of alkaloids in thinner roots.

Microscopical examination of the roots of *Datura* also reveals that in thinner roots the xylem occupies a small area in comparison to the total space which is mostly occupied by parenchymatous cells of the cortex Microchemical reactions with Wagner's reagent show the presence of alkaloids in these parenchymatous cells and this accounts for a larger percentage of alkaloids in thinner roots. In older roots, the amount of cortical tissue is less in comparison as the xylem tissue occupies more space and idioblasts of sandy crystals of calcium oxalate and starch fills in a large number of parenchymatous cells thus occupying the spaces which are the seat of alkaloids in thinner roots. This gives a probable explanation as to the cause of deficiency of percentage of total alkaloids in thicker roots

Our observations and probable explanation agrees very well with the view advocated by Moore (1947) in case of Derris roots where he found that the starch-bearing tissue predominated in thick older roots and that the resin-cell

tissue that contained the rotenoids was relatively more abundant in roots of small and medium diameters.

Summary

The roots of the plants *Rauwolfia serpentina* L. and *Datura metel* L. having the lowest diameters show the maximum percentage of total alkaloids which is found to vary inversely according to the diameters of the roots.

Acknowledgements

The author wishes to express his indebtedness to Dr. M. D. Chakravarti, Director, Central Drugs Laboratory for his kind interest and to Sri H. N. Rai Chaudhuri for his kind assistance.

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PHARMACOGNOSTIC STUDIES ON COMMERCIAL SAMPLES OF CINNAMON BARKS SOLD IN THE INDIAN MARKET

by S. C. Datta *and* D. Datta, *Central Drugs Laboratory, Calcutta*

(Communicated by B. Mukerji, F.N.I.).

A survey of the different samples of Cinnamon barks sold in the Indian Market shows that they differ widely in quality, aroma, pungency and oil content Literatures on the identification of these barks from pharmacognostic characters is scanty and nowhere complete informations on these points are available, although some workers as Dymock (1892-93), Fluckiger & Hanbury (1879), Gathercoal & Wirth (1947), Hooker (1890), Santos (1930), Trease (1952), Wallis (1946) and Youngken (1943) have reported the characters of some species of Cinnamon barks. Our observation showed that these barks belonged to different species of *Cinnamomum* and they could be classified into three groups, viz., (1) *Ceylon cinnamon,* consisting of barks of *Cinnamomum zeylanicum* Nees. and *C. pauciflorum* Nees., (2) *Cassia cinnamon,* consisting of barks of *C. iners* Reinw. and *C. tamala* Nees.; and (3) *Saigon cinnamon* consisting of barks of *C. obtusifolium* Nees. The pharmacognostic characters of these barks have been described in this paper and the distinguishing characters have been presented in a tabular form for purposes of identification of the samples

Materials

A large number of samples of Cinnamon barks were purchased from the local market and more than 100 samples were consulted during preparation of this paper. For comparison, genuine samples of *C. zeylanicum, C. tamala* and *C. iners* were obtained from Indian Botanic Gardens, Calcutta through the courtesy of Director. Samples of *C. pauciflorum* were obtained from Assam and barks of *C. obtusifolium* were supplied by the Curator, Industrial Section, Indian Museum, Calcutta.

Experimental

For pharmacognostic examination, the samples were studied under a simple microscope in order to observe macroscopic characters. Microscopic characters were studied from sections, macerated materials and powders. For preparing sections the samples of barks were softened by boiling in water and were kept in 70% ethyl alcohol. A few dried barks were also sectioned for studying histo-chemical reactions. The powder of the samples were obtained by drying the barks at 100° C, powdering and passing through a No. 60 sieve. Macerated preparations were made by boiling small pieces of bark with a mixture of equal volumes of 10% Nitric and Chromic acid, washing thoroughly in water, staining in dilute safranuin and mounting in 50% glycerine Microscopic measurements were done with the help of ocular and stage micrometer and the drawings were done with camera lucida.

Observations

The pharmacognostic characters of the different samples of barks are described below.

(1) *C. zeylanicum—Macroscopic characters.*

The outer surface is devoid of suberous coat and often some parts of the middle region in many commercial samples, light brown in colour, smooth or rough, finely striated with shining undulated lines and often with branched scars or holes. The inner surface is somewhat dark brownish in colour occa-

sionally with black or greenish spot. It occurs in several overlapping quilled pieces arranged carefully one within other, each side being made to curve inward forming a somewhat cylindrical structure with a groove along one side Fracture is very weak, brittle, splintery and uneven. Odour is fragrant and strong, taste is that of saccharin and aromatic, pungent. The commercial samples are usually found in sticks about 100 cm. long and 1 cm in diameter. The barks composing the sticks have a thickness of 1 mm. or less.

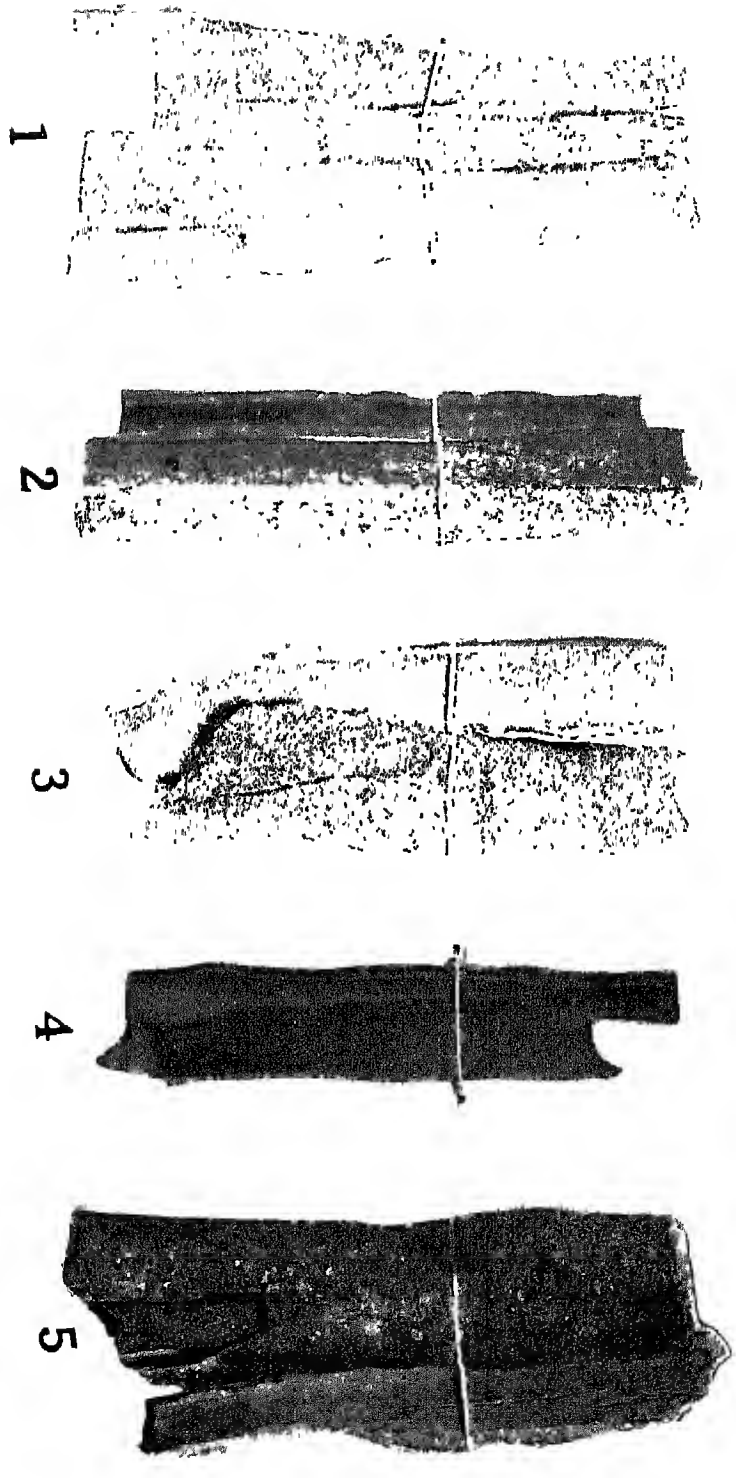
Microscopic characters.

In fresh young samples corks consist of 5 to 10 layers of thin-walled slightly suberised and tangentially flattened cells. In samples which have been cured for medicinal uses, this layer is absent. The phellogen and phelloderm layers are not distinct. The secondary phloem is composed of different types of cells and the outer region is characterised by the presence of 10 to 16 layers of parenchymatous cells containing starch Near the phelloderm tissue a number of sclerides and secretion cells containing mucilage or essential oil is present These secretion cells are also distributed throughout the whole region. The middle region is characterised by a continuous band of stone cells 3 to 4 cells wide. On the outer region of which 6-15 pericyclic fibres occur in group The stone cells are rounded, thick-walled, pitted and sometimes contain starch grains The inner region next to the stone cell layer is formed by 6 to 10 layers of parenchyma, which are thin-walled and tangentially elongated. The cells are often interrupted by secretion cells containing essential oils and the larger secretion cells contain mucilage. The parenchyma cells towards the phloem region are smaller and are traversed by medullary rays which are mostly 2-cells wide. They are isodiametric with thin wall and are filled with starch grains or crystals of calcium oxalate or both. The bast region constitutes nearly half of the entire section and is made up of phloem, phloem parenchyma, bast fibres, secretory cells and medullary rays 1—2 cells wide Towards the stone cell ring the phloem cells are often found to be collapsed into strands and the cell cavity is scarcely visible but near the cambium region they appear tangentially elongated. Phloem parenchyma cells are rectangular or rounded and contain starch grains, tannic acid or calcium oxalate crystals Conspicuous bast fibres are scattered throughout this region and have very thick walls and greatly reduced cavity. The oil secretion cells are also scattered throughout this region. The microscopic measurements of secretion cells are 25-90 u long and 12-30 u wide, stone cells 20-35 u X 30-50 u; cortical cells 15-25 u X 50-75 u

(2) *C. pauciflorum*—*Macroscopic characters.*

The outer surface is rough with somewhat longitudinal striations, greyish brown in colour with scar marks. The inner surface shows pale greyish brown colour, smooth in texture and similar to that of *C. zeylanicum*. The bark occurs in curved pieces although occasionally they occur in single quill. Fracture strong, brittle and uneven Odour similar to *C. zeylanicum* but less fragrant. Taste slightly sweetish, less pungent and somewhat slimy. The commercial samples are 10-15 cm. long and 3-4 cm. wide and the barks are about 2 mm. in thickness

Microscopic characters.—The cork cells are often present and usually consist of 10-15 layers of tangentially elongated cells The walls of the cork cells are not very much thickened. The cork layers are usually broken up in different parts of the section and at several places The phellogen layers are often found to be distinct and the cells are formed with 3 or 4 layers of very compressed thin cork cells. The phelloderm layers are not very distinct Next to the phelloderm layers the cortical tissues are composed of 20-30 layers of parenchymatous cells a number of which are somewhat tangentially flattened.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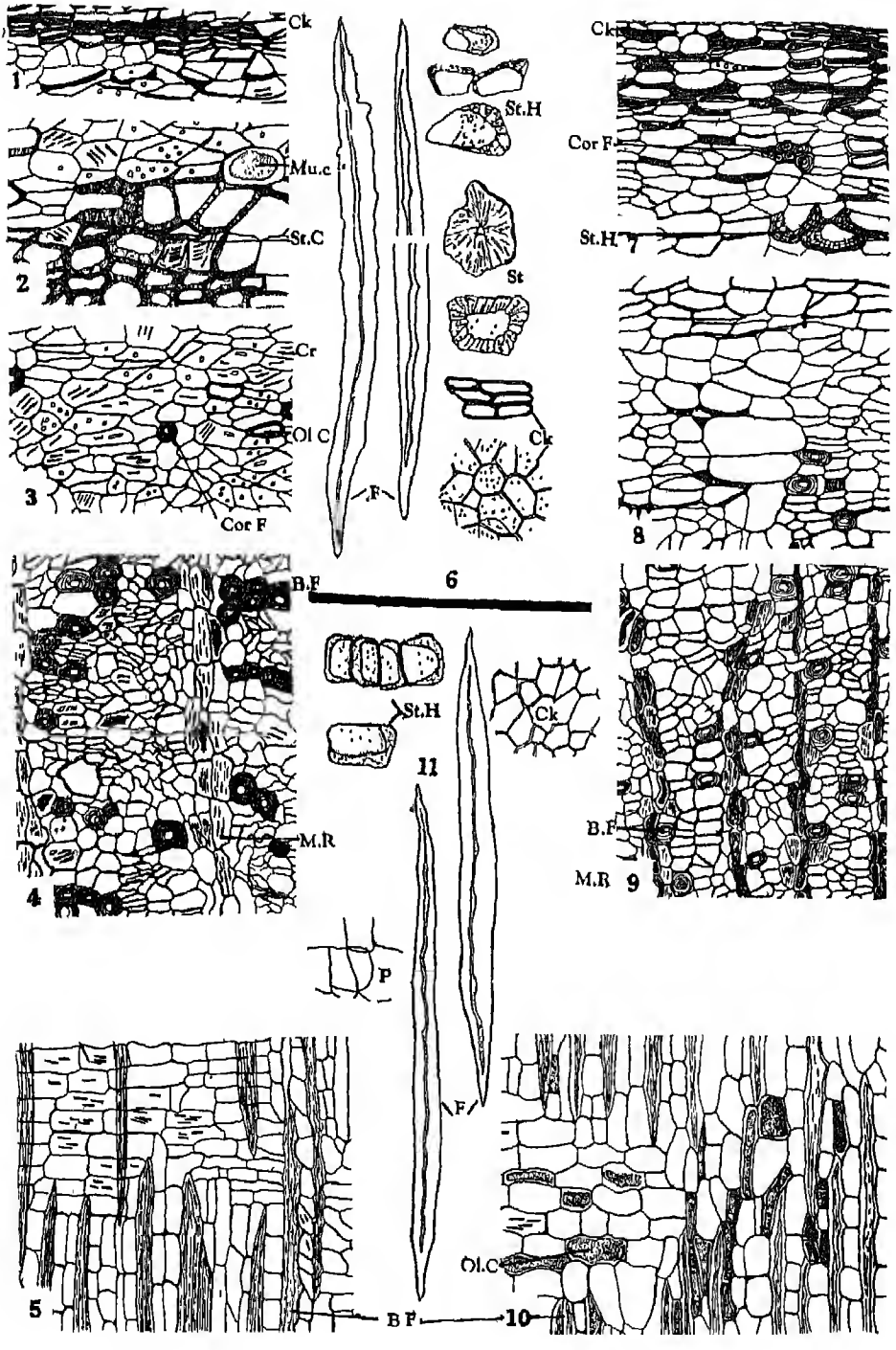
The outer region of the cortex is characterised by conspicuous stone cells which have one side thickened. Such half stone cells are abundant in the outer region but do not occur in the inner region of the secondary phloem. A number of secretory cells of which the larger ones contain mucilage and the smaller ones contain oil are also present in the cortex. In the middle region, inner to the cortex there is a stone cell layer 3-5 cells wide which is broken at places by strands of parenchymatous tissues. In the outer region of these stone cells small groups of pericyclic fibres occur. The secondary phloem region is characterised by the presence of medullary rays which appear to be conical, i.e. wide near the stone cells but narrow towards the inner part Bast fibres which are characteristic elements in other cinnamon barks in the secondary phloem region are almost absent in this species. Few secretory cells containing oil and mucilage also occur in this region and the major part of the tissue consists of parenchymatous cells. The measurements of the different elements are, secretory cells, 50-100 u X 90-125 u, bast fibres 20-30 u in diameter and 220-500 u in length, stone cells, 25-70 u X 50-75 u; parenchyma cells 35-40 u X 50-60 u

(3) *C. iners.—Macroscopic characters.*

The outer surface is light brown with greyish white or yellow-greenish patches of lichens or algae. It is uneven and rough with slightly elevated rounded or oblong lenticels and with transverse and longitudinal fissures on one side. Inner surface is reddish brown, slightly rough or granular The barks are usually curved, i.e, the curvature is slight or in simple quill. Fracture tough, uneven and slightly splintering in the inner region Odour aromatic and fragrant Taste astringent and slightly pungent. Pieces are sold usually in pieces of 10-15 cm. long and about 2—3 cm wide thickness of the bark varies from 2—4 mm

Microscopic characters.—The cork cells are composed of 10-15 layers of tangentially elongated slightly suberised cells all of which contain a reddish brown substance. The outer layer of these cells are generally thin walled and the inner ones possess thick, pitted and colourless wall. The phellogen and phellodorm layers are somewhat distinct. The cortical region is composed of several layers of small polygonal and tangentialy elongated cells filled with starch grains and calcium oxalate crystals Few secretory cells containing essential oil are scattered in this region. Outer region of cortex contain a number of stone cells. The stone cells are present in large numbers although generally they do not form a continuous ring as they are separated by tangentially elongated parenchymatous cells which contain starch grains and calcium oxalate crystals. The inner part of the pericycle is composed of parenchymatous cells in which there are few secretion cells containing either mucilage or essential oil. The bast region occupies the entire half part of the bark and is traversed by numerous medullary rays the cells of which contain crystals of calcium oxlate. The bast fibres present in this region are also numerous and are either isolated or are in radial or tangential groups with distinct striations The secretion cells are somewhat smaller in size and fewer in number than *C. zeylanicum*. The phloem cells are in somewhat collapsed condition. The medullary ray cells are filled with starch grains, calcium oxalate crystals or a brownish substance. Bast fibres are very prominent and are distributed throughout in transverse section. The measurements of the different elements are as follows—bast fibres, 100-200 u long and 5-10 u in diameter; stone cells 15-60 u X 10-30 u; secretion cells, 20-50 u X 10-20 u; cortical cells, 25-50 u X 45-60 u.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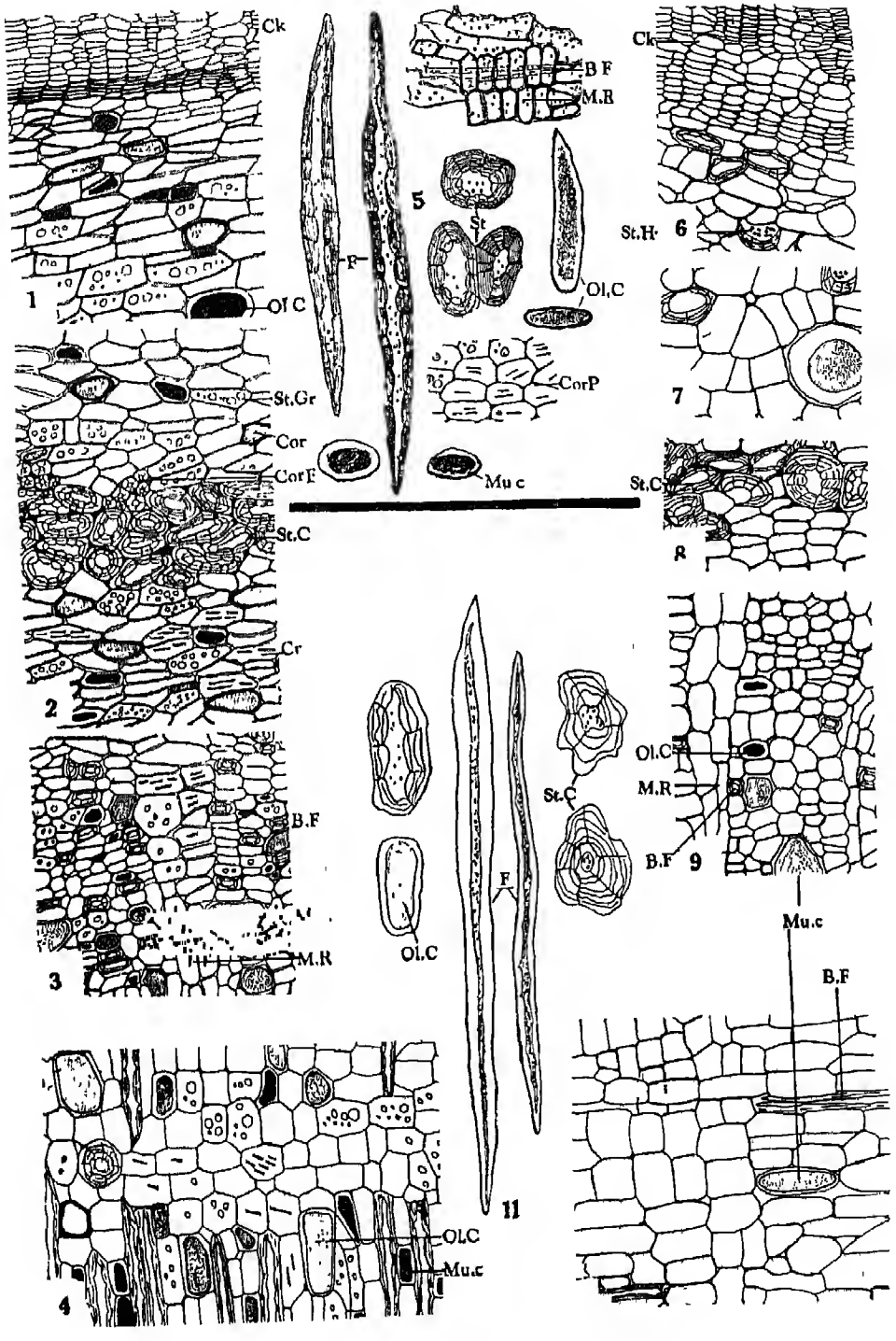
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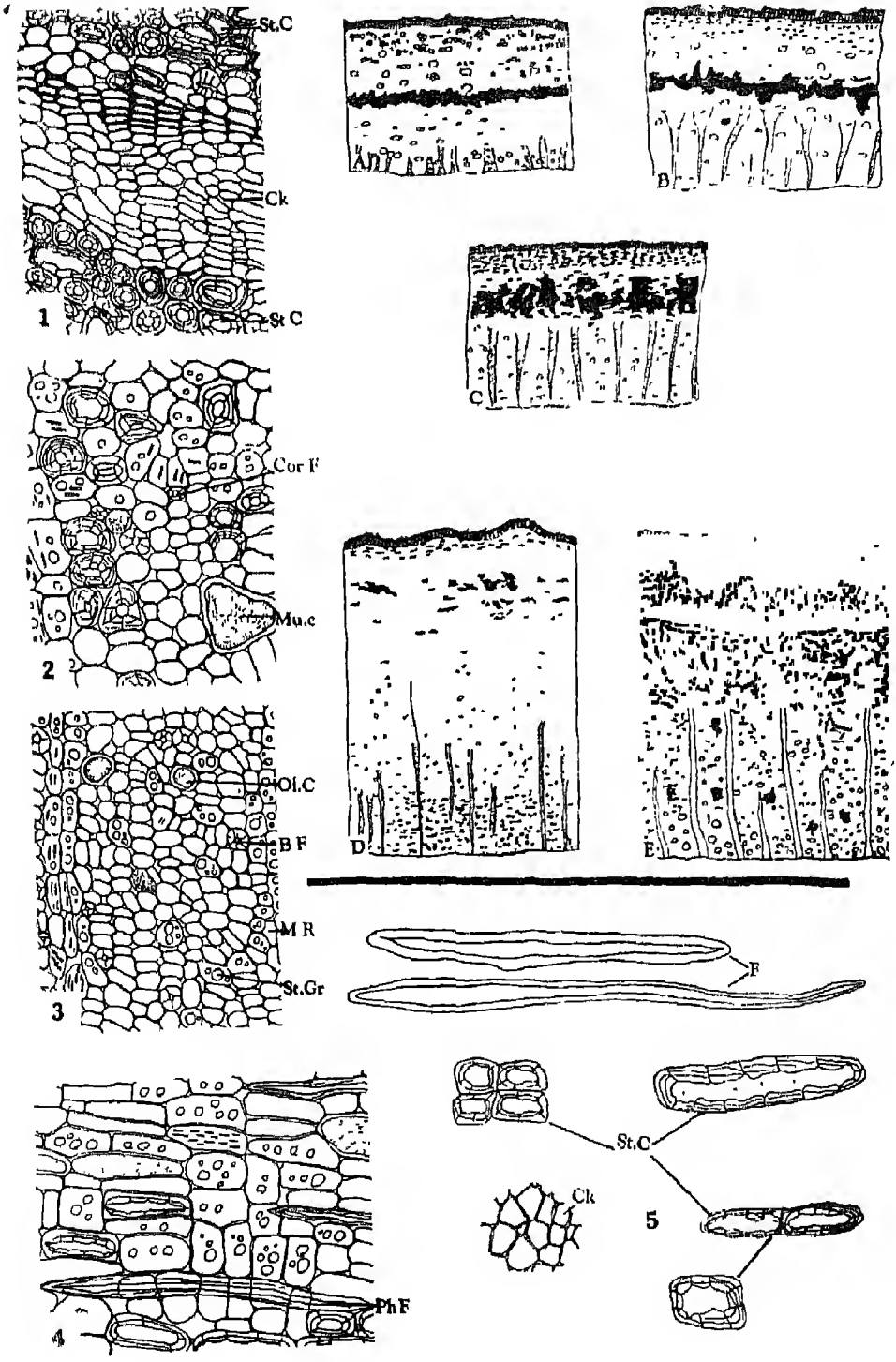
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(4) *C. tamala—Macroscopic characters*

The outer surface is greyish brown in colour, rough and somewhat granular, occasionally with presence of transverse marks or undulation with lenticels. Lichens are sometimes present. The inner surface is smooth and light brown in colour Curvature is slight, although sometime single quills are also sold in the market Fracture similar to *C mers* Odour similar to *C. zeylanicum* but less aromatic. Taste similar to *C. zeylanicum* The commercial samples are 8-12 cm. or sometime 20-25 cm. in length and 3-5 cm. wide The thickness of the barks vary from 3 to 5 mm.

Microscopic characters.—The cork consists of several layers of tangentially elongated cells the walls of which are not very much thickened They often fall off and are sometimes absent in commercial samples. The phellogen layer is somewhat distinct due to the presence of brownish content in the cells. The cortical tissue next to phellogen is characterised by the presence of 16-20 layers of parenchymatous cells which are filled with starch grains. Stone cells frequently occur in the outer region of the cortex generally in groups and are also distributed throughout the cortical region. Next to this region there are large groups of stone cells which do not actually form a continuous ring but are more or less arranged in a scattered way In the cortical region there are secretion cells containing oil, mucilage or tannin. Next to the stone cell layer the secondary phloem is characterised by the presence of different cell types and it occupies nearly three-fourth or more of the entire area of the bark. This region is characterised by the presence of numerous medullary rays generally 2-celled wide and contain abundant amount of starch. Large secretion cells containing mucilage and smaller secretory cells containing oil are distributed in the lower region of the secondary phloem either singly or in groups. Calcium oxlate crystals are present is some cells of the secondary phloem especially in the medullary rays The measurements of the different elements are as follows—stone cells, 30-50 u X 50-60 u; bast fibres 35-40 u in diameter and 350-550 u in length; cortical parenchyma 80-85 u X 125-150 u, mucilage cells 85 u X 190 u, oil cells 80 u X 90 u.

(5) *C obtusifolium—Macroscopic characters*

The outer surface is dirty greyish brown with presence of lichens, algae, dust etc. and many lenticels The surface is rough and characterised by the presence of both longitudinal and transverse fissures The inner surface is reddish brown in colour and rough The barks occur in curved pieces or double quills. Fracture short, hard, uneven, splintery. Odour faint, slightly aromatic. Taste slimy very slightly bitter, sweet and very astringent. It is sold in the market in pieces about 20-30 cm. long and the barks are 5-8 mm. or more in thickness.

Microscopic characters.—The transverse section of the bark shows more than one layer of cork cells. The walls of the cells of the outer layer of cork are thin and slightly suberised while those of the inner layer are generally thick. Next to the outer region of the cork a stone cell layer intervene between the outer and the inner cork layer. The stone cell layers are either more or less continuous or broken up into different strands. The tissue between the corky layers contain parenchymatous cells Cortical parenchyma, next to the corky layer is made up of several layers of parenchymatous cells and contain sclerenchymatous tissue in abundance. These cells occur either in groups or singly and gradually extend to the periphery of the secondary phloem region. These stone cells are distributed throughout the cortex uniformly and are not confined to special zones as in the case of other cinnamon barks The secondary phloem region consists of different types of tissues and occupy nearly the half of the area of the bark. The medullary rays are usually one or two-cells

wide and the walls of these cells are somewhat thicker and filled with starch. Large secretion cells are present throughout the secondary phloem and calcium oxalate crystals are also present. Few cortical fibres are embedded along with the stone cells in the cortical region Bast fibres are also present in the secondary phloem region. The dimension of the different elements are as follows; stone cells, 35-40 u X 200-250 u; secretory cells 60-75 u X 30-35 us; bast fibres 20-25 u in diameter and 250-300 u in length.

The distinguishing characters of the barks are given in the following Table I.

TABLE I.

Distinguishing Characters of Cinnamon Barks.

Characters	*C. zeylanicum*	*C. pauciflorum*	*C. iners*	*C. tamala*	*C. obtusifolium*
1. Thickness	1 mm. or less	2—3 mm.	2—4 mm.	3—5 mm.	5—8 mm. or more
2 Taste	Strongly pungent, aromatic and sweet	Slightly sweet less pungent & somewhat slimy	Astringent & slightly pungent	Sweet & aromatic, less pungent	Slimy, very slightly bitter, sweet and very astringent
3. Cork	Thin-walled, tangentially elongated	Same as *C zeylanicum*	Same as *C. zeylanicum*	Same as *C zeylanicum*	Alternate layers thin and thick celled cork
4. Phellogen	Not distinct	Distinct	Distinct	Distinct	Distinct
5. Half stone cells	Absent	Present	Present	Present	Absent
6 Stone cell layer	Continuous	More or less continuous or broken	Broken	Scattered	Widely scattered
7. Pericyclic fibres	Present in groups on outside of stone cell layer	Same as *C zeylanicum*	Distributed in the cortex	Distributed in the cortex	Distributed in the cortex

SUMMARY

The paper deals with the pharmacognostic characters of the commercial samples of Cinnamon barks sold in the Indian market and aims at finding out the identifying characters of the barks of *C. zeylanicum* Nees, *C pauciflorum* Nees, *C. iners* Reinw *C tamala* Nees and *C obtusifolium* Nees, all of which are sold under the common name Cinnamon Barks.

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Explanation of Plate.

Plate I.

1. Photograph of the bark of *C. zeylanicum* X 4/5.
2. Do Do *C. iners* X 4/5
3. Do Do *C. tamala* X 4/5.
4. Do Do *C. pauciflorum* X 4/5.
5. Do Do *C. obtusifolium* X 4/5.

Text Fig. 1.

A. Transverse section of the bark of *C. zeylanicum* (diagrammatic)—X 20.
B. Do Do *C. pauciflorum* Do —X 20.
C. Do Do *C. iners* Do —X 20.
D. Do Do *C tamala* Do —X 20.
E Do Do *C. obtusifolium* Do —X 20

1. Transverse section of the bark of *C obtusifolium* showing the alternate layers of stone cells and cork X 320.
2. Transverse section of the same showing the upper portion of the cortex X 320.
3. Transverse section of the same showing the secondary phloem region X 320
4. Radial longitudinal section of the same showing the secondary phloem region X 320.
5. Some isolated elements of the same X 320.

Text Fig. 2.

1. Transverse section of the bark of *C. zeylanicum* showing cork and upper portion of the cortex X 320
2. Transverse section of the bark of *C. zeylanicum* showing the cortical portion X 320.
3. Transverse section of the bark of *C. zeylanicum* showing phloem X 320.
4. Radial longitudinal section of the bark of *C. zeylanicum* showing phloem X 320.
5. Some isolated elements of the same X 320.
6. Transverse section of the bark of *C. pauciflorum* showing cork and upper portion of the cortex X 320.
7. & 8. Transverse section of the bark of *C pauciflorum* showing the upper and lower portion of the cortex X 320.
9. Transverse section of the bark of *C. pauciflorum* showing secondary phloem X 320.
10. Radial longitudinal section of the bark of *C pauciflorum* showing secondary phloem X 320.
11. Some isolated elements of *C.pauciflorum* X 320.

Text Fig. 3.

1 Transverse section of the bark of *C iners* showing cork X 320
2. & 3. Transverse section of the bark of *C iners* showing upper and lower portion of cortex X 320.
4. Transverse section of the bark of *C. iners* showing phloem X 320.
5. Radial longitudinal section of the bark of *C. iners* showing phloem X 320.
6. Some isolated elements of the same X 320.
7. Transverse section of the bark of *C. tamala* showing the upper portion of the bark X 320.
8. Transverse section of the bark of *C. tamala* showing the cortical portion X 320.
9. Transverse section of the bark of *C tamala* showing the cortical portion X 320.
10. Radial longitudinal section of the bark of *C. tamala* showing phloem region X 320.
11. Some isolated elements of the same X 320.

B.F., Bast fibres; Cor, cortex; Cor F., Cortical fibres; Cr, Crystals; F, fibres; M.R., medullary rays; Mu. C., mucilage cells; P, parenchyma; Ol. C, oil cells, St. C., Stone cells; St. H., Half stone cells, St. gr., starch grains

NEED FOR THE CULTIVATION OF DRUG PLANTS

by S. L. NAYAR, *Central Drug Research Institute, Lucknow.*

(Communicated by B. Mukerji, F.N.I.)

It is now felt by all concerned that there is a definite need for the cultivation of drug plants in India. The most important bottleneck which has so prevented India from bringing the pharmaceutical industries of India to a high order and from securing a world market in crude drugs is the utter neglect of quality control and the collection of crude drugs from wild sources which do not give a uniformity of product. There is either intentional or unintentional adulteration due to lack of proper knowledge on the part of the collectors, and collection is done without taking into consideration the proper season, time of collection, stage of growth and other factors. There is a considerable range in the amount of the active constituents in the wild plants growing in a state of nature and there also occurs a good deal of hybridation with allied forms resulting in the decrease of the active principles. It is also now felt that our natural resources of many drug plants are fast being depleted By the partition of the country a great set back has also come due to the fact that certain areas rich in medicinal plants have now gone to Pakistan. It is also well known that during emergencies like war great difficulty is experienced in getting supplies of drugs from other countries and the prices go up beyond the means of the people. If the cultivation of drug plants is taken up in a scientific way and on commercial basis, with intensive cultural methods it is practically possible to grow in India almost every drug ranging from those growing in tropical to temperate climates due to the wonderful variability of temperature, soils, and climatic conditions that the country possesses Due to this suitability of climatic and edaphic factors, it is possible to acclimitise here many exotic plants which do not grow here, and thus with very few exceptions, India can produce almost all drugs both for export as well as for home consumptions. But before the cultivation of drug plants on scientific lines can be undertaken on a commercial scale, research is necessary on the manifold aspects of this specialized industry.

It augurs well for the country that our Government has also realized the need for this type of work and has started an Indian Medical Plant Committee under the auspicies of the Indian Council of Agricultural Researeh, who are opening six centres for trial experiments for the cultivation of drug plants in different zones of India It is gratifying to note that schemes for the cultivation of drugs are under active consideration and are receiving the serious attention of the Uttar Pradesh Government in the district of Almora and by the Bengal Government in some parts of the Nilgiris The Himachal Pradesh Government has also under consideration such projects for sometime past. A number of medicinal plants already grow in Himachal Pradesh in a state of nature and the edaphic, climatic and altitudinal conditions met within its boundries render it particularly suitable for the cultivation of a large number of medicinal plants. With its wide range of climatic conditions varying from sub-tropical to temperate and alphine at different altitudes from 3,000-20,000 ft. and more, the excellent fertile soil under the rich forests, and the different amount of rainfall, it should be possible to make it a real drug house in point of requirement of crude drugs. The Kashmir Government has also made a beginning by starting an experimental drug farm in the State and important drugs like Belladonna, Digitalis, Hyoscyamus, Podophyllum and Pyrethrum etc. are being cultivated.

In one of the joint publications of the author, entitled 'Scope for the cultivation of medicinal plants in India (Chopra et al 1948 to 1949) the following 31 important drugs were discussed in detail as worthwhile for cultivating in various regions of India. 1. *Aloes* 2. *Anethum graveolens* 3. *Artemisia* 4. *Anacylus pyrethrum* 5 *Atropa belladonna* 6. *Cassia angustifolia* 7. *Cephaelis ipecacuanha* 8. *Chrysanthemum cinerariaefolium* Vis. 9. *Cinchona* 10. *Colchium autumnale* 11. *Claviceps purpurea* 12. *Digitalis purpurea* 13. *Ephedra gerardiana* 14. *Eugenia aromatica* 15. *Eucalyptus* 16. *Glycyrrhiza glabra* 17 *Hyoscyamus niger* 18. *Hyssopus officinalis* 19. *Ipomoea purga* 20. *Lavandula officinalis* 21. *Lobelia inflata* 22. *Mentha* species 23. *Olea europoea* 24. *Prunus serotina* 25. *Piper cubeba* 26. *Rhamnus purshiana* 27. *Rosmarinus officinalis* 28. *Strophanthus kombe* 29. *Theobroma cacao* 30. *Styrax benzoin* 31. *Valeriana.*

Besides these, there are some additional drugs worth cultivating in the country and they are as follows.

1. *Acacia senegal* Wild. The plant is a thorny tree about 10 to 15 ft. high and yields the gum arabic which is official in the pharmacopoeia of British and U.S.A. The plant is found to a small extent in south-east Punjab, northern Aravally hills and other parts of Rajputana The plant can be easily cultivated in the dry and arid tracts of Rajputana and Central India, and does not require any special attention after it has grown The tree is quite hardy and survives under most adverse conditions and grows on the poorest kind of desert soils. There is a great need at the present of growing forests in Rajputana in order to put a halt to the spread of the desert towards the North and to fight soil erosin. This tree will serve this purpose very well and in addition will yield the gum arabic which is of commercial importance. The average annual yield of gum from young trees is about 900 grams and from older trees about 2,000 grams.

2. *Aconitum chasmanthum* Stapf. The plant is a biennial herb and grows in a state of nature in the alpine and sub-alpine regions of western Himalayas, in high plateaus between 7,000-12,000 ft. The plant is included in the Indian Pharmacopoeial List as source of the drug Aconitum which consists of the dried roots of the plant. It is used internally in fevers and externally for neuralgia and rheumatism. The plant grows well on a peaty or leafy-mould soil containing a little lime. Elevated situations, some shade, cool climate and well drained gravelly loam are ideal conditions for its cultivation. This can be cultivated in the temperate Himalayas from 7,000-12,000 ft. and in alpine regions in Kashmir, in Darjeeling and particularly in Himachal Pradesh.

3. *Citrullus colocynthis* Schrad. The plant is a perennial herb. The dried pulp of the fruit constitutes the drug colocynth which is a very powerful hydragogue cathartic. The plant is found wild throughout India. The plant thrives very well in sandy loam semi-desert soils. It does not require any special method of cultivation as it grows rapidly after the seeds have been sown. This plant can also very well be cultivated in the dry and arid tracts of Rajputana and Central India to prevent sand drifts and at the same time will yield a useful drug.

4. *Coriandrum sativum* Linn. The plant is an annual herb indigenous to Italy, but is widely cultivated in Central and Eastern Europe, the Mediterranean and India Russia, Holland and Thuringia are important producers. The aromatic seeds and the oil distilled from them are used medicinally as a flavouring agent and carminative The yield of the volatile oil as well as its quality is variable in the different strains The yield of the oil is maximum i.e. 0.8 to 1 per cent in the Russian *C. sativum* The Indian Coriandrum, which is oblong in contrast to spherical fruits from other countries, yields the

lowest amount of volatile oil i.e. 0.15 to 0.2 per cent which is much below the pharmaceutical standard of other countries It is no use to continue the cultivation of such a poor variety in India and it is definitely worthwhile trying seeds from Russia and Germany to replace the Indian variety

5. *Datura innoxia* Mill. The plant is an annual herb indigenous to Mexico, but now grows freely in India The leaves and flowering tops are used in medicine. The only alkaloid present in this drug is hyoscine which has come to great prominence since the world war II for its effectiveness in the treatment of shell shocks and other neurotic diseases. The plants grow readily from the seeds and can be easily grown on rich clay loams and thrives in sunshine. The seeds give a much greater percentage of germination if subjected to alternate freezing and thawing before germination or treated with 2% solution of Hydrogen peroxide for 18-22 hours.

6. *Duboisia myoporoides* R. Br. and *D. leichhardtii* F. Muell The plants are small sized trees which grow wild in the sub-tropical regions of New South Wales, Australia. The leaves of these plants are very rich source of hyoscine and contain 4 to 5 times the quantity of hyoscine than in any other plant The Bureau of Plant Industry in Australia now recognize these trees as a very practical source of the drug hyoscine and are carrying on selection of suitable types rich in active principles from among the wild plants for its cultivation. These trees are capable of adapting themselves to a very wide range of climatic conditions and deserve special attention for trial cultivation in various parts of India.

7. *Foeniculum vulgare* Mill. The plant is a tall aromatic perennial. The fruits are used in medicine, by virtue of the volatile which they contain, for their aromatic and carminative properties. The plant is cultivated in Germany, Holland, Austria, Hungary, Bulgaria, Roumania, Russia, Italy, South France, North America and India. It is known to occur in a number of varieties The Russian and Saxony varieties contain 4 to 7% of the volatile oil which contains 22% of the principle known as fenchone for which the drug is valued In India *Foeniculum panmorum* DC. is cultivated and is considered only a variety of the official plant. The oil content of the Indian fennel is very small being 0.72% and its fenchone content is 6 7%. It would be very much desirable and profitable to introduce into India the better variety of this drug.

8. *Helopsis longipes*. This plant is indigenous to Mexico and deserves introduction in India on account of its insecticidal value. It contains an active principle known as scabrin which is a yellow viscous oil extracted with low boiling petroleum ether. This is two and a half times as toxic as pyrethrin to the house flies.

9. *Holarrhena antidysenterica* Wall The plant is a tall shrub or small tree indigenous throughout the plains of India except in the dampest districts, ascending to 4,000 ft. in the Himalayas. It is very common in the Dun and Saharanpur forests and is sometimes planted. The dried stem bark of the plant is known as Kurchi and is used in medicine principally as a remedy for amoebic dysentry. In recent years there has been a revival of interest in the drug in this connection and there is a good demand for it. So far this drug has been collected from wild sources and it would be worthwhile to pay attention to its cultivation.

10. *Plantago psyllium Linn.* The plant is a herbaceous annual indigenous to the countries of the Mediterranean region The seeds are used in medicine as an aid in cases of chronic constipation. The plant is grown on a commercial scale in a number of European countries. In India *Plantago ovata* is cultivated which has a swolling factor lesser than *P. psyllium.* The trial introduction of the latter species in India is, therefore, worthwhile.

11. *Podophyllum emodi* Wall The plant is an erect herb The dried rhizomes and roots constitutes the drug Podophyllum which is used for the preparation of the resin which is a drastic, but slowly acting purgative The plant is found in the interior ranges of the Himalaya from Hazara and Kashmir to Sikkim. The plant can be cultivated on higher altitudes, where the soil is rich and where there are shady temperate forests There is every chance that its cultivation in Sikkim Himalayas will probably meet with great success It can also be tried at altitudes of 9,000-14,000 ft. but it may give good results even at lower altitudes between 6,000-9,000 ft. The plant deserves a special [illegible] respect.

[illegible] *serpentina* Benth. The plant is a small erect shrub found in the sub-Himalayan tracts and in the plains near the foot of the hills from Sirhind eastwards to Assam, especially in Dehra Dun, the Siwalik range, and in the sub-Himalayan tracts of Rohilkhand, north Oudh and Gorakhpur, ascending to an altitude of 4,000 ft ; also in Konkan, North Kanara, southern Mahratha country, Western and Eastern Ghats of the Madras State, many districts of Bihar such as Patna and Bhagalpur, and in north and central Bengal. It is the roots of the plants which are used in medicine for their well-marked hypnotic and sedative properties, and for reducing the blood pressure. In recent years considerable interest has been taken in this drug and at present there is a very great demand for it from foreign countries So far this drug has been collected only from wild sources and already the supply has become very short. The roots from the Dehra Dun variety yield 1-1.3 per cent total alkaloids and clinical trials show that the extract prepared from these is more effective in reducing the blood pressure than from roots from other parts of the country. The cultivation of this drug deserves special recognition and attention to it is urgently called for.

13. *Urginea maritima Baker.* The plant is a perennial herb indigenous to the Mediterranean region. The dried sliced bulbs of the plant constitute the drug squill. The drug has a digitalis like action on the heart and in small doses is used as an expectorant. In large doses it is emetic. The plant is largely cultivated in Malta, Italy, Algiers and Sicily on the sandy soil near the coast. Planted from seed, it requires 5 or 6 years to reach marketable size, whereas 4 or 5 years are necessary if bulblets are used. The yield per acre every 5 or 6 years is about 20,000 to 30,000 pounds. It is worthwhile trying its cultivation on the sandy seashores of India and also in the sandy soil in Kangra valley where Indian squill i.e. *U. indica* grows in a state of nature.

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A BRIEF SURVEY OF ADULTERATED CRUDE DRUGS OF INDIA

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(Communicated through U. P. Basu, F.N.I.).

In the Indian market many drugs are sold which are adulterated and are unfit for medicinal use There are many forms of adulterations (Bal, 1951), viz.,—(i) Sophistication, (ii) Admixture, (iii) Substitution (iv) Deterioration, (v) Spoilage and (vi) Inferiority. In a recent survey carried out by this

Institute, it was revealed that during 1952-53, out of the total number of important pharmaceutical crude drugs analysed, 48% were genuine and of good quality, 39% were of low-standard and 13% were altogether spurious drugs. During previous year, i.e., 1951-52, the figures were 55% genuine, 25% low-standard and 20% spurious.

It is not the object to discuss here all the types of adulterations found in the drug trade of India, but only one type—i.e. the sophistication which will be discussed in this paper. Sophistication means the addition of spurious articles to any material or supplying wholly spurious articles with intent to defraud There were many sophisticated drugs which are so alike, that, detailed examinations were required to find out the true nature of the sample. Some of the spurious drugs discussed here, have already been noted and published (Bal 1952; Datta 1948; Datta, Gupta and Bal 1948; Datta, Rao and Datta 1949; Gupta and Bal 1952; Melville 1949) There are many other adulterants which have not yet been reported and the trade and commeree are not much aware of their presence The botanical origin of those drugs which do not contain flowers or fruits, can not be given, as no information about the source of the drugs are available from the trade. To specify them only trade names or coined names were given. Those spurious drugs which have not yet been reported and discussed in short, will be published elsewhere in detail in future. The spurious drugs were collected from Calcutta, Bombay, Delhi and other important markets of India.

Belladonna Folium

It is known to the pharmacognosists that due to high price of the drug, it is often sophisticated with Datura leaves, *Scopolia* sps. and some other Solanaceous plants. During the course of investigations a type of sophisticated leaf was detected whose anatomical nature as an adulterant is not found in the literature. Some of the samples contained the same spurious leaves, but they were not found in piled condition with the genuine drugs, i.e., the leaves of *Atropa belladonna* and *A. acuminata* The spurious samples do not contain flowers, fruits or seeds They are often called in the commerce as "Belladonna Leaf No. 2", and are found in all the principal drug markets of India, specially at Bombay. Obviously these spurious leaves are supplied from the same source to these places. In Table I a short note on distinguishing characters are given along with the two official varieties of "Belladonna herb or leaf"

Table I (Belladonna Leaves)

	A. belladonna (Kashmir)	*A. acuminata* (Kashmir)	Belladonna leaf No. 2. (Spurious)
Flowers	Brownish	Yellowish brown	Absent
Fruits	Dark	Brownish to greenish	Absent
Diameter of stems	Less than 5 mm.	Less than 4 mm.	Often more than 10 mm
Leaves-colour	Dull green, lower side paler	Brownish green	Deep green on both surfaces
Texture	Slightly rough	Slightly rough	Almost smooth
Hairs	Nearly glabrous	Slightly hairy	Glabrous
Crystals	In minute clusters	In minute clusters	Accicular crystals in bundles on surfaces as white dots.
Shape	Broadly ovate	Oblong-elliptic, tapering at ends.	Ovate to oblong.
Size	5-25 x 2.5-12 cm.	5-15 x 2.5-6 cm	10-25 x 4-10 cm.
Apex	Acuminate	Acuminate	Acuminate.
Margin	Entire	Entire	Entire
Petiole	0 5-1 0 cm	1.0-2.0 cm	4-10 cm.

Belladonnae Radix

A few samples were obtained which contained some roots which are anatomically different from the officially recognised roots of *Atropa belladonna* and *A. acuminata*. These roots are sometimes wholly substituted or are piled with genuine ones The wholly substituted roots are sold as Belladona Root No. 2''. These spurious belladonna roots when subjected under ultra-violet light, the woody portions do not exibit the characteristic bright blue radiation, that is found in the official species. The organoleptic descriptions of the two official species of *Atropa* and those of the spurious root, are given in the Table II

Table II (Belladonna Root)

	A. belladonna (Foreign, commercial)	*A. acuminata* (Kashmir)	Belladonna Root No. 2" (Spurious)
Crown	Present, with stem bases	Present, with stem bases	Absent
Root colour (external)	Weak brown to greyish	Dark brown	Greyish brown
Root colour (internal)	Whitish	Yellowish	Brownish yellow
Shape	Entire or cut longitudinally, almost cylindrical, not twisted	Entire or cut longitudinally almost cylindrical, not twisted	Entire, rarely cut longitudinally occationally splited, twisted .
Thickness	0.5—1—3 cm.	0.5-1.5-3 cm	0.3-1.6-2 cm
Periderm	Abraded	Almost intact	Thin, easily separable
Wrinkles	Fine, longitudinal	Coarser, longitudinal	Roughly longitudinal or fissured
Fracture	Smooth, mealy	Short, splintery	Very tough & fibrous
Inner wood	Mealy, cambium zone dark	With conspicuous concentric rings	Fibrous with concentric rings
Longitudinal splitting	Difficult, uneven	Difficult, uneven	Very easy, surface even
Taste	Sweetish, then bitter & acrid	Sweetish then bitter & acrid	Bitter, acrid, little mucilaginous
Odour on boiling	Peculiar & narcotic	Peculiar & narcotic	Herbaceous

Buchu Folia

There is a type of leaf sold in the market under the name ''Indian Buchu'' which is spurious in nature. Buchu is derived from the leaves of different species of *Barosma* and this genus is not represented in India. Also the plants are not known to be introduced in any part of the country. The spurious Buchu of the market is found either in homogenous state or is piled with genuine Buchu leaves. It has got almost same colour and texture which make it easy to pile with genuine one. There are three official species of Buchu,—*Barosma betulina* (Round), *B. crenulata* (Oval), and *B. serratifolia* (Long), which are all found in South Africa. Among these *B. betulina* or 'Round Buchu' is most commonly imported in this country. The organoleptic characters of these official Buchu and the spurious one are tabulated in the Table III.

TABLE III (Buchu Leaves)

	B. betulina (Round Buchu)	*B. crenulata* Oval Buchu)	*B. serratifolia* (Long Buchu)	*Indian Buchu* (Spurious)
Shape	Rhomboid-obovate	Oval to oblong	Linear lanceolate	Ovate-elliptic to linear
Size in mm	12-20 x 4-15	15-30 x 7-10	20-40 x 4-10	10-50 x 4-8
Apex	Blunt, recurved	Blunt but not recurved	Truncate	Acute
Margin	Dentate to serrate	Minutely serrate	Serrate	Entire
Surface	Punctate	Punctate	Punctate	Smooth
Oil glands in lamina	Small glands all over	Small glands all over	Small glands all over	Absent
Oil glands at crenatures	Present, small glands	Present, small glands	Present, small glands	Absent
Oil glands at apex	One large gland	One large gland	One large gland	Absent
Odour	Strong, characteristic	Strong, characteristic	Strong, characteristic	Mild, aromatic
Taste	Sharp	Sharp	Sharp	Cardamom-like

CHIRATA HERBA

Chirata is a very common article sold in the retail crude drug shops. Adulteration in Chirata is widely spread (Bal 1952) and the most common adulterants are different sps. of *Swertia* and *Andrographis*. Also it is said that all sorts of bitter herbs are sold as Chirata. Th[illegible]onsists of the upper parts with flowers and fruits of the plant [illegible] (Roxb.) Lyons. The common adulterants are *Swertia alata, S. angustifolia, S. trichotoma, Andrographis paniculata* and *A. echioides*

The most distinguishing features among commercial *S. chirayita* and *Andrographis* sps. are as follows: Chirata has a peculiar yellowish tinge all over the herb in dried state, the stem is faintly quadrangular at the top an cylindrical below. The cauline leaves broad at the base. The flowers are borne in panicles, which are 2-3mm. broad and ovoid in shape; the fruit is a capsule which is ovate, bicarpellary In case of *Andrographis* sps. ('Kalmegh'), the stem is strongly 4-winged, mostly solid, the colour of the leaves are narrowed at both ends, 3-6 pairs of secondary veins emerge from the mid-vein. Flower is Acanthaceous, small, capsule linear upto 17 x 4mm. in commercial samples with rugose seeds.

Among the inter-generic species of *Swertia,* the herb of *S. chirayita* distinguished by the presence of two glandular depressions near the base of each of the corolla-lobes, flowers 4-merous, and the pith is large, separable and somewhat dark in colour with intensely bitter taste

Digitalis Folia

In India most of the home consumptions of the Digitalis leaves were met with *Digitalis purpurea* grown in Nepal. A small quantity is also grown in Kashmir and Darjeeling area. Recently the import of the drug from Nepal reduced considerably and consequently there is an acute shortage of this important drug in India. The trade is now importing a large quantity of "Spanish Digitalis" (*Digitalis thapsi*) from Europe thinking it to be genuine and official. But this Digitalis is an adulterant and should not be used in the official preparations. Also it has been reported that *Lobelia* leaves and *Verbascum thapsus* were sold as Digitalis. The distinguishing features are given in the Table IV.

Table IV.

Digitalis Leaves (Commercial)

	D. purpurea	*D thapsi*
Colour	Leaves deep green, lower surface paler	Leaves yellowish-green to brownish
Texture	Papery	Velvety
Foreign matters	Almost absent	Contains much foreign matters like grasses, smal stems, dirts, etc.
Leaf size	10-30 x 4-10 cm.	5-15 x 1.5-5.0 cm.
" shape	Ovate-lanceolate	Lanceolate to ob-lanceolate
" margin	Crenate to serrate	Serrate to dentate
Venation	Highly reticulate, in lower surface much prominent, raised & veins paler	Reticulate and not so prominent in lower surface where it is darker & covered with wooly trichomes
Hairs	Non-glandular hairs much more than glandular	Wooly, non-glandular hairs absent
Vein-islet No. per sq mm.	2 to 5.5	8.5 to 16

Lobelia Herba

Official Lobelia (Datta, Rao & Datta 1949) is derived from *Lebelia inflata* which is an American plant. Some times past it was found that among various species of Indian Lobelia, only *L. nicotianaefolia* was found to be the most desirable substitute. *L. nicotianaefolia* is a South Indian plant but also grows near Bombay. Consignments came also form other parts of India which aroused suspicion and on examination of these samples three spurious forms were isolated which were subsequently identified and compared withthe herbarium of Indian Botanic Garden, Calcutta. The species forms were *Lobelia pyramidalis, Eclipta alba* and *Fuga ramosissima*. The characters of all these species are given in the Table V.

TABLE V.

Lobelia

	L. inflata	*L nicotianaefolia*	*L pyramidalis*	*Eclipta alba*	*Laggera aurita*
Stem	Pubescent, cylindrical to furrowed, with purplish patches.	Pubescent, yellowish brown, pith wide often hollow, stems not more than 5 mm.	Glabrous, greenish yellow with pink in patches or all over pink, pith wide, stems 2-10 mm. broad	Hirsute, purplish brown, elliptic to flat, pith wide, stems upto 7 mm.	Villous greyish green, cylindrical, pith narrow, stems upto 3 mm
Leaf texture	Slightly pubescent.	Pilose, not crumpled, mostly broken.	Almost glabrous, crumpled and broken.	Hirsute, crumpled & broken.	Hirsute, much crumpled and broken.
Leaf colour	Yellowish green.	Green lighter below.	Green.	Dark green.	Light green.
Leaf shape	Ovate to oblong.	Narrowly ovate lanceolate.	Linear lanceolate.	Ovate, variable	—
,, size	3-8 x 2-3 cm.	5-10 x 2-5 cm.	10-18x2-4cm.	2-4 x 0.5-1 5	Small.
,, margin	Obtusely dentate.	Finely serrate.	Serrate.	Entire.	Toothed
,, petiole	Subsessile	Subsessile	Nearly petiolate.	Subsessile.	Slightly decurrent.
Flower	Plenty, bluish racemes, campanulaceous	Much, pubescent, campanulaceous.	Few, glabrous campanulaceous	Scanty, villous, compositae	Scanty, small, villous compositae.
Fruits	Plenty, inferior.	Plenty, inferior.	Few, inferior	Scanty	Scanty.
Odour	Slight.	Tobacco like.	Tobacco-like.	Aromatic.	Aromatic.
Origin	N America*.	S. India.	C. & E. Himalaya.	Bengal Plains.	Bombay & U.P.

PRUNUS VIRGINIANA CORTEX

Wild cherry bark or Pruni Virginiana bark is derived from the rossed older bark or unrossed younger bark of *Prunus serotina* which is a plant of North America. Both the above mentioned types of bark are regular articles of trade. For sometimes back a type of bark has come to the market under the name of "Indian Pruni Virginiana whose botanical source is unknown to pharmacognosy. This bark has some similarity with rossed bark of *Prunus serotina*. The macroscopic characters of the type is given below.

Indian Pruni Virginiana : Most barks are longitudinally curved and the pieces are of various dimensions The outer surface is variable in characters. Some are scarlet in colour, glossy with several layers of glossy, foliaceous corky layers, some blackish scarlet, crustacious, rough (unrossed) outer surface, some

* Successfully grown at Darjeeling but not yet grown commercially.

are bright brown (rossed), smooth to little wrinkled surface Lenticels are absent in all forms. Inner surface brown to blackish-brown, the striations are more longitudinal, some fissures are present which are wider than those of true species. The inner phloem region is light brown with some radial dark coloured specks. Thickness varies from 0.5 to 12 mm. Odour sweet and unlike that of bitter almond, taste bitter and astringent.

Senega Radix

The different forms of spurious senegas were discussed by the writers in a paper (Gupta & Bal, 1952). The important diognostic features of two genuine (*Polygala senega* and *P. chinensis*) and four spurious ("Delhi", "Kullu", "Tuticorin Yellow", and "Tuticorin Brown-green"—the origin of which are unknown) forms are given in the following table No. VI

Table VI. (Senega Root).

	P. senega.	*P. chinensis*	Delhi.	Kullu.	Tuticorin Yellow.	Tuticorin Brown green.
Colour.	Orange-red	Brown.	Dirty brown.	Greyish black.	Yellowish.	Reddish with green under-surface.
Shape.	Large crowned.	Crowned, twisted angular.	Large crowned.	Tuft of clipped stems, no crown, thin.	Shrivelled, crowns small.	Dense, crowns big.
Rootlets.	Much.	Few.	Absent.	Filliform attached.	Absent.	Absent
Root branching.	Spreading.	Drooping.	Unbranched.	Drooping.	Unbranched	Unbranched.
Root diameter.	4-6 mm.	3-5 mm.	5-8 mm.	3-5 mm.	3-5 mm	4-7 mm.
Bark.	Thick, easily separable.	Thin, separable.	Thick, easily separable.	Thin, unseparable.	Thin, unseparable.	Thin separable
Taste	Bitter, acrid, sternutatory.	Slightly acid sternutatory.	Indistinct.	Slightly acrid.	Bitter, acrid, sternutatory.	Slightly acrid
Fracture.	Sharp, smooth.	Sharp.	Brittle, smooth.	Tough, splintery.	Brittle & splintery.	Splintery.
Secondary Phloem.	Wide, radial.	Radial.	Radial.	Narrow, radial.	Scattered.	Wide, scattered
Keel.	Prominent.	Present.	Absent.	Absent.	Absent.	Absent.
Maximum diameter of vessels.	63μ	50μ	75μ	34μ	63μ	70μ
Growth rings.	Indistinct.	Prominent, 3-5.	Indistinct.	Distinct, 5-8	—	Indistinct.
Starch.	Absent.	Present.	Present.	Absent.	Abundant.	Present.
Wood-fibres.	Absent.	Abundant.	Few.	Absent.	Absent.	Present.

Strophanthus Semina

Strophanthus seeds are important cardiac drug and all of the official species of *Strophanthus* are African Though there are two or three species of *Strophanthus* native to India, yet little is known about their medicinal properties. Medicinally important species of *Strophanthus* are *S kombe, S. hispidus* and *S gratus* None of these are native to India but it was found by the writers (Gupta & Bal 1952) that *S. hispidus* is growing wild, near about Calcutta, the seeds of which were subjected to pharmacological tests in the laboratory of this Institute and were found to contain sufficient potency. Also *S. wallichii* (Indian) was tested similarly, but it was found to contain no activity. The reported adulterant (Melville 1949) of the seeds is the seeds of *Holarrhena antidysenterica* (Kurchi seeds or 'Indrajab') The important identifying characters are tabulated below in Table VII

Table VII. (Strophanthus Seeds)

	S kombe (African)	*S hispidus.* (Indian)	*S wallichii.* (Indian)	*S dichotomous.* (Indian)	*H antidysenterica.* (Spurious)
Colour.	Greenish white.	Dark brown.	Yellowish brown.	Brown.	Brown.
Shape.	Lanceolate.	Ob-lanceolate.	Oblong	Linear lanceolate.	Linear lanceolate.
Apex.	Prolonged into awn.	Prolonged into awn.	Blunt.	Prolonged into awn.	Blunt, constricted below.
Trichomes.	Silky.	Coarser.	Glabrous.	Glabrous.	Glabrous.
Length in mm.	15-18-20.	7-12-16.	5-6-7.	15-23-30.	8-13-20.
Breadth in mm.	2 4-3.3-4.1	2-2.5-3.	1.3-1.8-2.2.	2 5-3.6-4.2.	2-3.4-4.
Thickness in mm.	0 9-1.3-1 8	1.1-1.3-1 5.	0.4-0.6-0.8	0 5-0.9-1.2.	1-2-3
Raphe/ length of the seeds.	Extending half.	Extending half.	Whole length.	Whole length.	Whole length.
Wt. of 100 seeds	3-4 gms.	1 3-1.5 gms.	0.3-0 35 gms	2.0-2 5 gms.	2-4 gms.

Viburnum Cortex

Viburnum bark is derived from the root and stem of *Viburnum prunifolium* and *V. rufidum* which are American plants. The barks are either collected from younger branches (thin variety) or are collected from thick branches (thick variety). Sometimes thick barks are 'rossed' before marketing. A bark which is now selling in the market in the name of "Indian Viburnum" was anatomically analysed and was found to be different in nature. The bark of 'Indian Viburnum' is almost similar in external appearance to the younger unrossed stem bark of *Viburnum prunifolium*. The obvious difference in longitudinal section was the presence of fibres in place of stone cells in the cortical and phloem regions in "Indian Viburnum".

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STUDIES IN THE PHARMACOGNOSY OF *TINOSPORA CORDIFOLIA* MIERS—(GUDUCHI)

by A. N. NAMJOSHI, *of the work done under the auspices of the Board of Research in Ayurveda, Bombay.*

(Communicated by B. Mukerji, F.N.I.).

Tinospora cordifolia Miers, in Ann. & Mag. Nat. Hist. ser. 2, v. 7 (1851) p. 38, & Contrib. v. 3 (1864) p. 32

Syn. *Cocculus cordifolius* DC Syst v. 1, p. 518; Grah Cat p. 4.

Family—Menispermaceae.

Names in Indian languages.

	Language.	*Names.*
1.	Sanskrit,	Guduchi, Amrita, Amrita-vallari, Chinna, Chinna-ruha, Chinnodbhava, Chinnangi, Tantrika, Kundali. Chakra-lakshinaka, Mandali
2	Hindi,	Gilo, Gurueh.
3.	Bengali,	Gulaneha.
4.	Marathi,	Gulvel.
5	Gujrathi,	Galo
6.	Canarese,	Amrytavalli.
7.	Konkani,	Amrit-vel, Garudvel.
8.	Malyalam,	Amrytu.

Tinospora cordifolia Miers, the plant under study has a great utility in Ayurvedic pharmacy and has been very extensively described in the various Sankskrit books of Ayurvedic Nighantoe [illegible] Bhavprakash Nighantoo, Ghana Nighantoo, Raj Nighantoo, [illegible] and others. Some of the very Sankskrit names given above are descriptive of the plant and have great pharmacognosical significance. The most common parts of the plant used are its stem and the contents of the stem, especially, "Guduchi-satva"—the starchy extract containing some unidentified presumably bitter principals.

DISTRIBUTION AND HABITAT.

It is a perennial climber belonging to the family Menispermaceae. It is growing all over India abundently in the thick or hilly forests under the shelter of evergreen trees and taking support of the forest trees of all dimensions. It is a climber, which, though initially starts a terrestrial life can live indepen-

dently of the tap-root, once it spreads in air and bears aerial adventitious roots.

Due to its extraordinary capacity to grow adventitious roots its propogation by cuttings, layerings etc. is very common and takes place artificially as well as naturally.

External Morphology.

Tap-root.—The tap-root of the plant could be procured with a little difficulty as the plant spreads from tree to tree getting its moisture requirements through the profuse adventitious roots, hanging in the air or from the soil through many adventitious roots which reach the soil and establish secondary relations with it.

The tap-root is a cylindrical structure a few feet in length, tapering towards the end, branching and bearing root hairs. The upper part of the root is rhizomatic in structure while the lower one exhibits typical dicotyledonous characters including the characteristic radial arrangement of the xylem and phloem.

Adventitious roots.—As stated above the plant can strike adventitious roots practically from any part of the stem They appear as thin and fleshy thread like structures with a root-cap, hanging down from the plant and gradually thicken and grow downwards till they touch the soil where they branch and enter the soil

The thread-like cylindrical adventitious roots are usually greenish yellow in colour tending to shining grey, due to the development of velamen tissue, away from the tip It is practically of uniform thickness, about 2 mm. in diameter The root is tipped with a root-cap with which it penetrates into the soil and then it branches laterally so as to fix up into the soil.

Stem.—It is a climber of the class Liana trailing and climbing from tree to tree without special organ of climbing. The stem is uniformly cylindrical in shape. The stem is glabrous, younger parts presenting pale green to brownish green appearance while the older part presents a brownish yellow rough surface studded with frequent lenticels oblong in shape and having a short vertical slit. Most of the adventitious roots originate from the lenticels. The phyllotaxis of the plant is alternate both for leaves and branches.

Leaves.—The leaves are simple, exstipulate, petiolate, cordate at the base with acute apex raised on long cylindrical petioles possessing a pulvinus. The margin is entire and the surface is rough and hairy, prominently on the dorsal side The lamina has multicostate(7) palmately reticulate venation, prominent on dorsal surface. It has a dark green colour on the ventral surface and yellowish green on the dorsal. The texture is leathery. The length of the leaf varies from 5.5 to 19 cms and breadth 6 to 20 cms.

Inflorescence.—The flowers are usually borne in May and June at the advent of rainy season and are unattractively greenish yellow coloured and small in size. It is a dioecious plant with male racemose inflorescence and solitary female flowers.

Male inflorescence.—Racemose, about 6-10 cms. in length, usually axillary.

Staminate flowers.—Bracteate (about 1 mm.), pedicellate (about 1-2 mm.) pale greenish yellow in colour.

Calyx.—Polysepalous 6, in two whorls of 3 each; sepals narrowly ovate with concave outer surface.

Corolla.—Polypetalous 6, cuneate, each embracing a filament at its base and reflexed at the apex.

Androecium.—Polyandrous 6, free, longer than the petals with fleshy filament and connectives; Anthers 4 lobed

Pistillate flowers.—These flowers are rarer and are seldom detected due to their solitary position and inconspicuous leaflike colour, resembling the male flowers in general appearance with trilobed ovary.

Fruit.—Drupes

Internal Morphology.

Transverse Section of Stem.—Dried pieces of stem being used as Ayurvedic drug, the adult stem; both fresh and dry were examined and it was found that the anatomical structures of the fresh as well as the dried sample are identical in their essentials except that the dried sample has its bark ruptured and wrinkled outwardly

(i) The transverse section of the adult stem is circular in outline with occasional projections where the section passes through the lenticels

(ii) *Bark.*—The outermost few layers from the bark are suberised and form a layer which is loosely attached to the cortex.

(iii) *Lenticels.*—The lenticels occur on the bark of adult stem and have vertical orientation

(iv) *Cortex.*—(a) The Chlorophyllous region occupies about 1/3rd of the cortex and is composed of about 4-5 layers of outer collenchyma cells and the subsequent 6-7 inner layers are formed of parenchyma. The collenchymatous cells are angularly thickened and contain chloroplasts and starch grains The parenchyma tissue is made up of thin-walled rounded cells containing chloroplasts and starch grains

(b) The non-chlorophyllous region of the cortex occupies 2/3rd of its area and is constituted of a couple of layers of rounded parenchymatous cells Immediately within these layers are 5-6 layers of radially elongated thin-walled parenchymatous cells some containing solitary cigar-shaped crystals of calcium-oxalate. This non-chlorophyllus region seems to store a considerable quantity of starch, as starch grains occur within the cells when no chloroplasts are present.

(v) *Pericycle.*—The pericycle consisting of [illegible] forms a characteristic layer of tissues, having a peculiar [illegible] by the naked eye. It forms a continuous circle of small crescent shaped arches outlining the vascular bundles and touching the adjoining arches of similar design. It consists of sclerenchymatous lignified cells forming 8-9 layers.

(vi) *Vascular Bundles.*—The vascular bundles in transverse section give the appearance like a complete wheel wherein the pericycle represent the rim, the central pith representing the axle and the medullary rays separating the porous vascular tissue as the spokes.

In a transverse section of adult stem about 14-24 vascular bundles could be counted with the naked eye. The vascular bundles are open, collateral and consists of conspicuous typical xylem and phloem elements. Crystals of calcium-oxlate are found in the phloem cells.

(vii) *Pith & Medullary rays.*—The medullary rays appearing like the spokes of a wheel lying between two adjoining vascular bundles radiate from the central pith. The pith occupies a small central portion and radiates in between the vascular bundles as medullary rays. The pith consists of thin-walled parenchymatous cells loaded heavily with starch grains. The medullary rays are formed of thin-walled parenchymatous cells slightly elongated radially and also heavily loaded with starch grains and occasionally possessing cigar-shaped crystals of [illegible]

Transverse Se[illegible] root.—The transverse section of an adventitious root presents a circ[illegible] exhibits a typical histological root structure. In the centre is [illegible] pith with polygonal paren-

chymatous cells. The pith is surrounded by a ring of four groups of vascular bundles, the phloem alternating with xylem. The xylem and phloem possess typical structural elements. Outside the vascular ring is a big region of thin-walled parenchymatous cells consisting of 5-6 layers containing chloroplast. This is encircled by a small ring of 3-5 layers of parenchymatous cells The outermost layer consists of 3-4 layers of velamen tissue for absorption of moisture from the atmosphere.

The tip of the advantitious root is covered with root-cap possessing typical tissue structure

"Guduchi—Satva".

In the Ayurvedic medicines the solid starchy extract of *Tinospora cordifolia* known as "Guduchi Satva" is very commonly prescribed. The "Guduchi Satva" is prepared by soaking pieces of dry or wet stem in water for 24 hours and beating them to a coarse pulp and levigating the same with water removing the fibrous matter, the cellulose matter, the chlorophyll and other light, soluble and insoluble substances. The heavy starchy matter which settles at the bottom is washed with water repeatedly till it becomes white Then it is finally mixed with water and allowed to settle The clear supernatent water carrying some light impurities is decanted out and the starchy sediment is dried in the air and stored as "Guduchi Satva"

In Ayurvedic pharmacopoeia "Guduchi Satva" has been described as a "Shitavirya" drug useful as a "Pitta-Shamak" medicine It is also described as febrifuge and a mild tonic. "Guduchi Satva"—therefore is in great demand in the market and the genuine stuff could be had at a price of about—Rs 8/- to 10/- per lb. There have been no test so far for identifying this starchy drug, except that it has a slightly bitter taste Adulteration of other starches has been found to be very common in the bazaar drug, which is sold under the name of "Guduchi Satva" or "Gilo Satva". The commercial brains being clever enough to go to the extent of adding some bitter substances to the maize, potato, wheat or rice starch and sell it as genuine stuff.

An attempt has been made in this work to lay down some method by which the genuine drug could be singled out from the spurious stuff and the percentage of adulteration if any could be assessed.

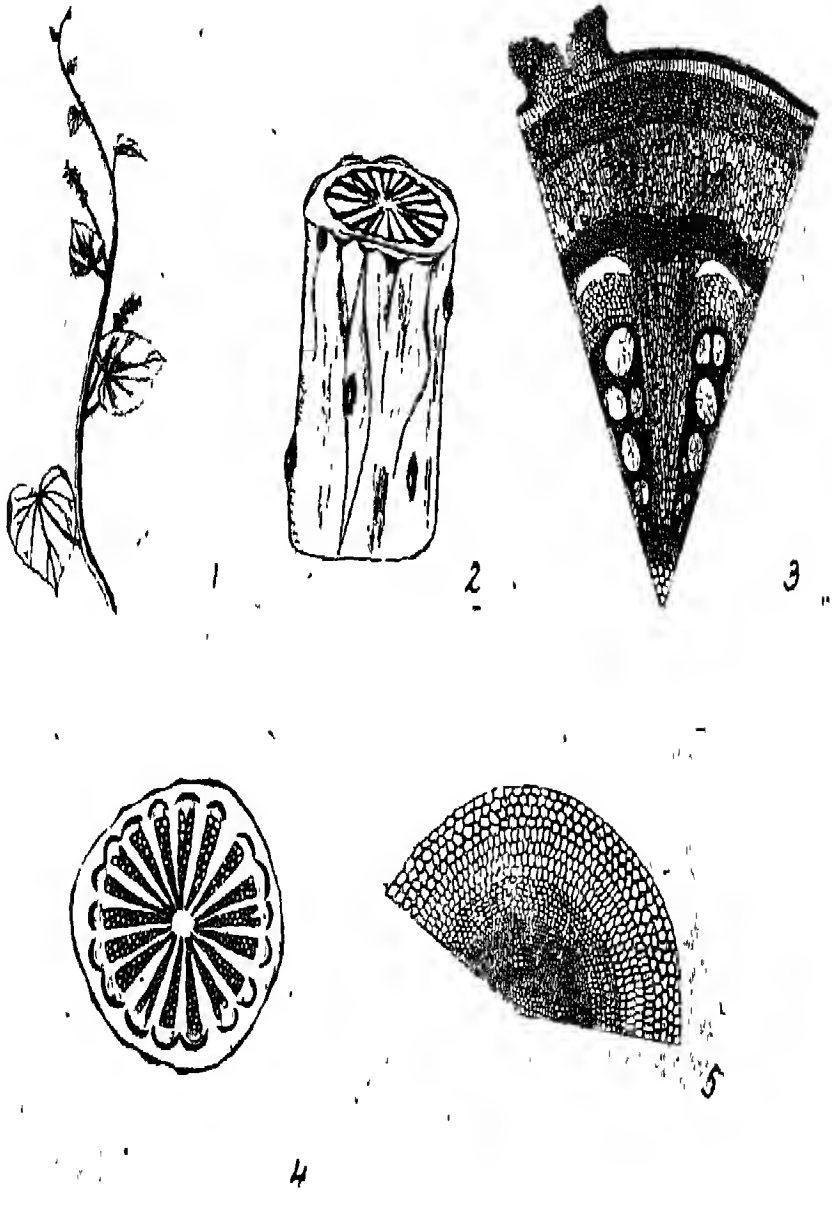
A microscopic examination of the starch grains of *Tinospora cordifolia* constituting the bulk of the drug "Guduchi Satva" was made with a corresponding comparative examination of other starches,—the likely adulterants such as wheat, rice, potato, Maize and Arrow-root starches The examination revealed one outstanding feature of starch grains of *Tinospora cordifolia*, namely their characteristic shape, as shown in micro-photographic reproduction.

The description of the starch grains examined under microscope is as follows:—

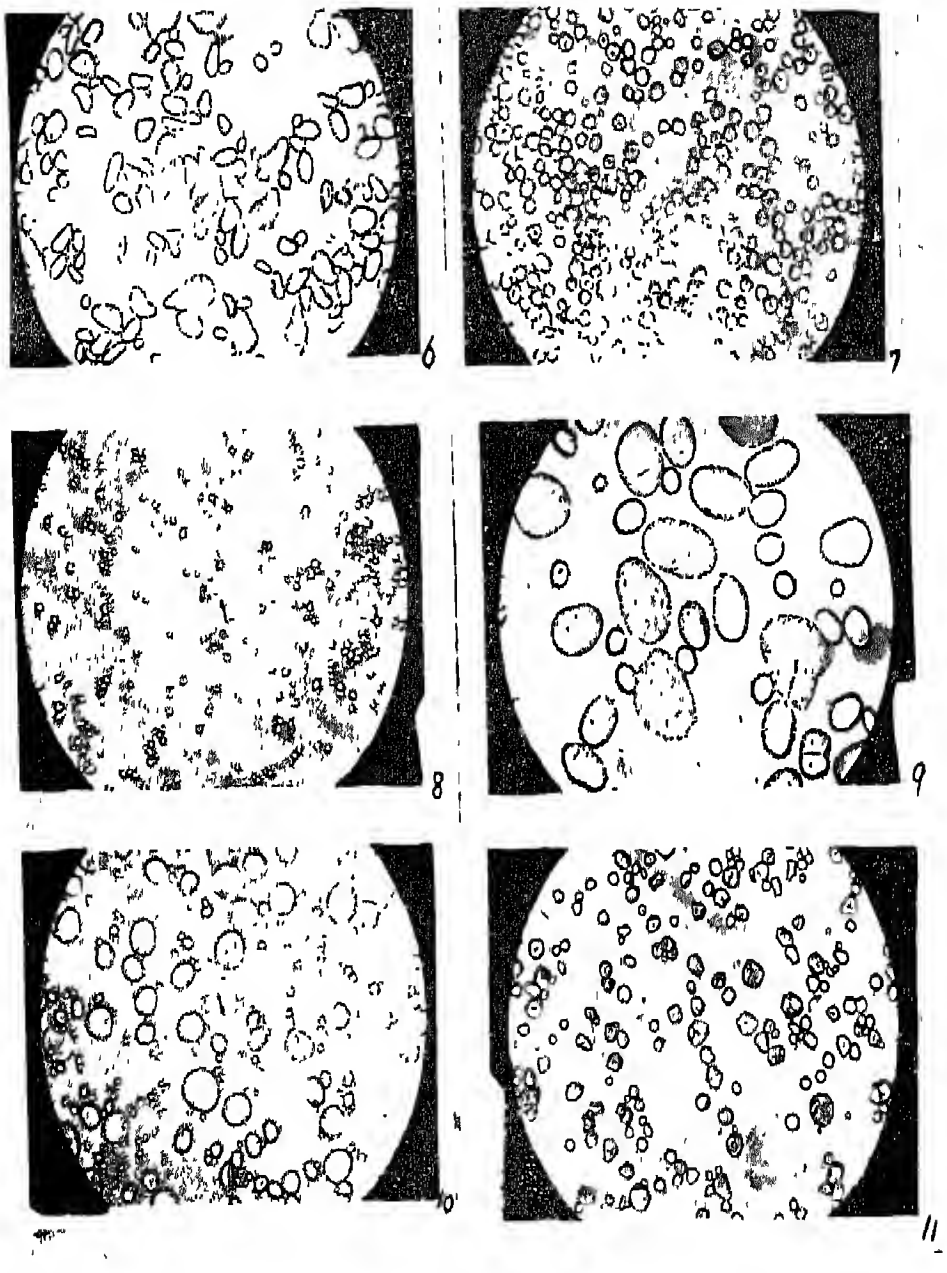
Shape.—Flattened, ovoid, irregularly triangular, sometimes tending to be lunate; some occasionally possessing roundish knob-like projection on the narrowed side.

Hilum and Striations.—The bigger starch grains usually exhibit striations though faintly and in that case they also present a hilum though not very conspicuous The hilum in such cases is excentric

Size.—The size of the starch grains varies greatly though the shapes are more or less characteristic The average length of the starch grains is 23 microns and average breadth is 15 microns The actual [illegible] 8 microns to 44 microns in length and from 4 microns to 2[illegible]



1 *Tinospora cordifolia*, Miers. 2. View of a piece of stem of *T. cordifolia* Miers 3. Sector of t.s of stem of *T. cordifolia*, Miers. 4 Gross view of t.s of stem of *T. cordifolia* Miers 5. Sector of t.s. of adventitious root of *T. cordifolia* Miers.



Micro-photographic Reproductions of.
6 *T. cordifolia*, Miers. Starch (Guduchi-Satva). 7 Maize starch. 8 Rice starch.
9 Potato starch. 10. Wheat starch. 11. Arrow-root starch.

Distinguishing Characters.

(1) Peltate leaves.
(2) Loose bark with lenticels having vertical orientation.
(3) Habit of producing adventitious roots through lenticels.
(4) The transverse section of adult stem is nearly circular in outline.
(5) A gross examination of the transverse section shows a continuous circle of crescent shaped arches of sclerenchyma outlining the vascular bundles, touching the adjoining arches of similar design and constituting the pericycle.
(6) A naked eye examination of the transverse section shows a design like a wheel possessing spokes, the pericycle representing the rim, the medullary rays the spokes and the pith the hub.
(7) *Microscopic examination of starch grains.*—The starchy matter when examined under microscope presents large sized starch grains possessing typical and characteristic shape which is not resembling with that of any common starch adulterant as seen in the microphotographic reproductions.

Note.

(i) It has been a traditional belief among the Ayurvedic practitioners that the "Guduchi Satva" obtained from Guduchi plant growing on "Neem" tree (*Azadirachta indica*, A. Juss) is more efficacious and the same is exploited by suppliers and the traders. With a view to verify this belief such a plant was procured and the morphological and histological structures of different parts of the same including starch grains were examined and compared with normal Guduchi Plant It was found that there is no morphological or histological difference whatsoever between the two The taste of the material as well as of the extract is also similar.

Unless otherwise proved the so-called observation that the "Guduchi Satva" from Guduchi, growing on Neem tree is more bitter and more efficacious sems to be due to the fact that the collectors or traders must have inadvertantly or wilfully adulterated some Neem stem (which is bitter and also medicinally useful) while extracting the "Satva" with a view to get the special price for their stuff.

(ii) Stem and leaf structures of *Tinospora rumphii*, Boerlage and *Tinospora reticulata*, Miers, have been described by José K Santos *Philippine J. Sci.* 1928, 35(2) 187) which are the species not occuring in this country and quite different in structure

MEDICINAL MALE-FERNS OF NORTH-WESTERN HIMALAYAS

by K. L. Handa, L. D. Kapoor *and* I. C. Chopra, *Drug Research Laboratory, Jammu & Kashmir Government.*

(Communicated by R. N. Chopra, F.N.I.).

Male-Fern is one of the oldest anthelmintic drugs known and which was used by the ancient physicians for expelling worms from the intestines of man and animals. Even now it is one of the best remedies against tapeworm and is administered in the form of liquid extract filixmas

The British Pharmacopoeia recognises the rhizomes and frond bases of *Dryopteris filixmas* (Linn) Schott. a fern indigenous to Great Britain as official for medicinal purposes. In America *D. marginalis* A Gray, which is found in Eastern and Central United States and North to Prince Edward Island forms the source of American Male-fern.

D. filixmas and *D. marginalis* are not indigenous to India but the other ferns belonging to the *Dryopteris* (Lastrea) *filixmas* complex grow in a state of nature in the Himalayas in general and in the mountainous ranges of Kashmir in particular. These ferns are —

Dryopteris rasthorni (Diels) C. chr.; *D. blanfordii* (Hope) C. chr., *D. odontoloma* (Moore) C. chr.; *D. ramosa* (Hope) C. chr.; and *D. marginata* (Wall) Christ., *D. brunoniana* (Wall) Kuntze.; *D. barbigera* (Moore) Kuntze., *D. levingei* (Clarke) C. chr., *D. robertiana* (Hoffon) C. chr.; *D. Phegopteris* (L) C. chr.; *D. serrato-dentata* (Bedd) Hayata., *D. repens* (Hope) C. chr.; *D. calcarata* (Bl) O. Ktze.; *D. schimperianum* (Hochst) C. chr.

Considerable quantities of the male-fern extract are annually imported into India for medicinal purposes In order to study if any of the above-mentioned ferns growing in the country can be substituted for the official ferns, samples of these were collected for the study of their active principles

D. odontoloma occurs widely in the fir zones forests as an undergrowth throughout the Kashmir Valley It is more common on the southern aspect of the forests retaining moisture at altitudes of 5,000 to 8,500 feet above the sea-level.

D. marginata is found at places with comparatively less moisture in the *Pinus excelsa* zone at altitude of 5,500 to 6,500 feet.

Five samples of *D. odontoloma* and one of *D. marginata* were collected from the various localities in Kashmir and the results are given in table I From the perusal of the results it is apparent that the local ferns are up to B.P. and U.S.P. standard so far as their active principles are concerned These can therefore be used for the preparation of the male-fern extract of B.P. standard for use as anthelmintic.

This work was further extended to some more ferns of *Dryopteris* species Specimens were also collected from the Western Himalayas from Kashmir, Chamba and Mussoorie areas in order to see if these could form suitable substitute for the official drug Their distribution is briefly summarised below.

Dryopteris blanfordii (Moore) C. chr.—This is a fern of the forest floor and is distributed in the Himalayas. It is found in Kashmir and Himachal Pradesh. Our specimens were collected from Chamba.

Dryopteris barbigera (Moore). O. Ktze.—This is abundant in the alpine meadows and in avalanche gullies. It is distributed in the Himalayas from Kashmir to Sikkim. This was collected from Gulmarg forests.

TABLE I.

Analytical Data of Ferns D. odontoloma and D. marginata.

Species	Locality	Calcium oxalate crystals	Percentage of total ash	Percentage of acid in soluble ash	Percentage of Filicin in the rhizomes	Percentage of extractive.	Percentage of Filicin in the extractive	Remarks
D. odontoloma	Gandherbal	Absent	3.6	0.51	2.8	10.9	25.6	Up to B.P. & U S.P. standard.
	Pehlgam	-do-	3 8	0.87	2 3	11.7	19.6	The fern is up to B.P. & U.S P. standard but the extract falls short of the standard.
	Bosian	-do-	3 2	0.44	3.1	11.4	27.1	Up to B P. & U S.P. standard.
	Bhedi	-do-	3.1	0.32	2.5	9.4	26.5	—do—
	Batot	-do-	4 2	0.78	2.1	7 9	26.5	—do—
D. marginata	Tanmarg	-do-	2 9	0.34	3.03	10.9	28 4	Up to B.P. & U S.P. standard.
	B P 1932 limits	-do-	Not more than 6%	Not more than 2%	—	—	24-26% W/W	
	U S P. XII limits	—	—	Not more than 3%	Not less than 1.5%	—	Not less than 24% W/W	

TABLE II.

Analytical Data of Ferns D odontolma and D. marginata

Species	Locality	Calcium oxalate crystals	Percentage of total ash	Percentage of acid in ash	Percentage of filicin in the rhizomes	Percentage of extractive with ether	Remarks
Dryopteris barbigera	Gulmarg (Kashmir)	Absent	2 3	0 12	2 1	7.71	Up to standards.
D. blanfordii	Chattari, (Chamba)	-do-	3.1	0 4	3.5	8 2	—do—
D. Schimperianum	Mussoori	-do-	2 8	0 24	4.4	13 3	—do—
D. calcarata	-do-	-do-	8 2	2.6	0 11	0.59	Not up to standards.
D. marginata	-do-	-do-	4 1	0.6	2.1	10.7	Up to standards.
D. odontoloma	-do-	-do-	3.5	0.31	2 3	9.2	—do—
B.P. 1948 standards	—	-do-	Not more than 6%	Not more than 2%	Not less than 1 5%	—	
U S P. XIII standards	—	-do-	—	Not more than 3%	-do-	—	

Dryopteris schimperianum (Hochst) C. chr—This is a common fern in Mussoorie and is found growing on ridges at altitudes of 7,000 ft It has tough sub-coriaceous dark green fronds and was procured from Mussoorie

Dryopteris calcarata (Bl). O. Ktze.—This fern is found in Mussoori area though somewhat sparsely; specimens of rhizomes of *D. odontoloma* and *D. marginata* growing in Mussoorie were also procured along with those of *D. schimperianum* and *D. calcarata*. Their analytical results are given in table II

Summary

D. filixmas official in British Pharmacopoeia and *D. marginata* official in the United States Pharmacopoeia are not found in India A number of closely allied species occur in this country which on investigation were found to come up to the standard for preparation of the extract for use as anthelmentic.

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AROMATIC PLANTS OF THE NORTH-WESTERN HIMALAYS

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(Communicated by R. N. Chopra, F.N.I.).

Introductory

Natural perfume is one of the most remarkable phenomenon of plant metabolism and it is not surprising that the history of aromatic plants is perhaps the most romantic story of any vegetable product. Man has always tried to increasingly utilize these odoriferous plants for his pleasure and well-being. From ancient times spices derived from aromatic plants have been used as flavouring agents for foods and drinks. Their use as offering to deities, as incense, in medicine, for aesthetic purposes, as principle agents for embalming the dead and for preventing insects from damaging fabrics and grain has been in vogue from times immemorial. From the early times particularly during Greek & Roman period, a large trade between India and the West flourished and even now some of the Indian aromatic plants products are greatly valued. Modern advance in science considerably added to our knowledge of the aromatic odoriferous substances. The separation of these principles in pure form from plant material led to their synthesis and production of artificial scents and perfumes. Whereas our ancestors had only a limited number of aromatic substances for their use, advances in chemistry have provided a very great variety of synthetic perfumes. Besides this many more are pleasant fragrant smells have been discovered and are being into use. Perfumed preparations such as hair oil, soap, skin creams, lotions, toilet powders, tooth paste, bath salts, smelling salts, etc. are increasingly in use and are on the market.

The demand for these aromatic substances has thus increased enormously during recent years. It is indeed surprising that with the exception of a few scents and *attars* produced in India, no serious attempt was made in this country to survey, record and study the natural source of perfumes, i.e. the aromatic plants growing in the Indian sub-continent and their large scale utilization in commerce and industry, in the growing soap and cosmetic industries. At the present times most of these perfumes are imported from foreign countries. It is gratifying to note that Essential Oil Committee (1946) of the Council of Scientific and Industrial Research has drawn attention to this subject and stressed the possibilities of its development. Krishna and Badhwar have put together the work so far done on aromatic plants in India, in a series of papers published in the Journal of Scientific and Industrial Research. Our interest in this subject was stimulated during the course of survey of medicinal plants of the North Western Himalayan Region. We were greatly struck by the profusion of aromatic plants growing in this area and by the richness and fragrance of odours given off by them.

Aromatic plants of Kashmir.—Preliminary survey of essential oil bearing plants growing in a state of nature in the Kashmir valley and on the bordering hills was systematically started by the workers of Drug Research Laboratory in 1945. Quite a large number of plants were collected and their essential oil contents were worked out. Our preliminary studies showed that these plants roughly fall into three groups.

1. The first group consists of well known plants whose essential oil content compared well with that of plants grown in other parts of the world. These can be taken up for commercial exploitation immediately with a little

plants either do not occur elsewhere or they have not been exploited so far for commercial purposes. These plants in their state of nature give small yield of essential oils but the contents could possibly be increased by proper cultivation and hybridisation.

III. Besides the plants in the above two groups on which preliminary work has been done, there are others in the North Western Himalayas which are less known and which are being studied These constitute the third group. In the following table a list of plants so far discovered is given

It has been stated that, as a general rule, fragrant flowers flourish in warm climates, but the more delicate perfumes are derived from plants having a colder habitat. India is endowed with all conceivible climates, seasons and soils and there is no reason why, most, if not all of the commercial perfumes could not be cultivated and developed in one part or other of our country There is no doubt that many of the aromtic plants discovered in this region could be cultivated and brought into use.

Cultivation of aromatic plants.—The cultivation of aromatic plants and extraction of perfumes is not difficult though enhancement of the essential oil and its particular aroma in the plant for which it is valued is a specialist's job. This has been done in case of lavender and peppermint in Miteham and Hitchin in England from where the best qualities unsurpassed anywhere are obtained

The introduction of some essential oil bearing plants both for pharmaceutical and cosmatic purposes has been attempted in Kashmir by the workers of the Drug Research Laboratory and the results so far have been encouraging The seeds in some instances were imported from abroad through the kind courtesy of UNESCO. The experimental observations are recorded as under:—

Name of Plant	Percentage of oil from locally grown plant	Percentage of oil from plant grown in U.K or Europe
Anethum graveolense	2.1	2.4 B.P.
Chenopodium ambrosioides var *anthelmonticum Gray* .. .	0 82 to 1.16	0 6 to 1 0 B.P C
Mentha piperita	0.7 to 1.0 on (dry leaves)	Not less than 0.50
Mentha pulegium	2.30	—
Ocimum kilimandscharicum ..	4 70	5 0
Lavendula officinlis .. .	2,4 dry flowers	0.8-1.7 fresh flowers

With modern advances in the science of breeding and selection, the development of exquisite aromas and greater yield of essential oils are matters which deserve careful attention. France is one of the most advanced countries in this respect. The cultivation of aromatic plants and the distillation of essential oils from them for purposes of perfumery is highly developed in that country The manufacture of essential oils in Grass in the South of France is perhaps the most important centre of perfumery dealings in the odorants On an average of about 62,50,000 lbs of flowers and leaves are distilled annually and essential oils produced are sent to all parts of the world.

There is no reason why such an industry should not be developed in India on a cottage scale industry in the first instance in the Himalayan belts where nature has abundance of aromatic plants. Such an industry could be greatly developed if necessary facilities in the form of technical advice, tariff protec-

blending or finer treatment in the final stages. They can meet the requirements of the consumer industries such as toilet, soap, hair oil and talcum powders manufacturers.

II. The second group includes local plants yielding essential oils; these

Name of Plant	% yield of oil from	
	Local specimens	Foreign specimens
GROUP 1		
Mentha sylvestris	1.20	0.90 (Cyprus)
Mentha arvensis	0.45	1.05 (Japanese)
Mentha piperita	0.71	0.50-1.00 (U.K.) 1.60-1 70 (Russia)
Mentha pulegium	2.30	0 60-1.70
Lavandula officinalis	2.40-3 00	0 80-1.70 (U.K)
Thymus serpyllum	0.72	0.15-0.60
Acorus calamus	3.10	1.50-3.50
Hyssopus officinalis	0.70	0.30-0.90
Angelica glauca	1.30	0.35-1.00
Elsholtzia cristata	0.93	2 00
Juniperus communis	0.77	1.00-1.50
Zanthoxylum alatum	2.01	3.70
Aegle marmelos	0.54	0.60
Archangelica officinalis (roots)	0.80	—
Archangelica officinalis (seeds)	3.80	0.30-0 90
Carum carvi	4.30-8.50	3.50-6.00
GROUP 2		
Inula racemosa	0.38	
Skimmia laureola	0.49	
Saussurea lappa	1.22	
Nepeta ciliaris	0.54	
Cinnamomum tamala	1.20	
Chaerophyllum villosum	0.98	
Salvia moorcroftiana	0.25	
S. glutinosa	0.32	
S. dumitorum	0 34	
S. hians	0.24	
Heracleum cachemericum	0 80	
Elsholtzia densa	0.98	
Ferula jaeschkeana (roots)	1.20	
Ferula jaeschkeana (seeds)	3 80	
Artemisia draconculus	0.70	
Juniperus macropoda	3.32	
Prangos pabularia	0.65	
Senecio jacquemontiana	1.20	
GROUP 3		
Nepeta ruderalis	Studies are in progress.	
N. elliptica		
Iris kashmiriana		
I. kumaonensis		
Betula utilis		
Pleotranthus rugosus		
Artemisia laciniata		
A. grata		
A. parviflora		
A. amygdalina		
Anthemis nobilis		
Origanum vulgare		
Achillea millefolium		

tion, marketting, etc., are provided. At any rate India could be made self sufficient so far as perfume for its soap and cosmetic industries are concerned in a very short time.

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CULTIVATION OF PYRETHRUM IN KASHMIR

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Introductory

Historical.—The systematic cultivation of pyrethrum plant in India, as a source of powerful insecticide, was taken up by the Forest Department of Kashmir State as early as 1931. Many unsuccessful attempts to grow the plant from imported pyrethrum seeds were made and it was not until 1934-35 that a small quantity of viable seeds were procured from Paris, which were sown and successfully germinated in Kashmir in due course. Incidentally Puntambeker outlined the possibilities of pyrethrum cultivation in Northern India and predicted that it had a bright future in this country The Forest Department of the State at once took up the idea and decided to take up cultivation right earnestly on a commercial scale.

Experimental cultivation was started in Baramulla Forest Nursery and seeds were sown in prepared beds. A fair percentage of seeds germinated which were carefully transplanted. These plants yielded some flowers and seeds were carefully collected for raising more plants. The germination percentage was much better than the previous year showing that the plant was probably getting adapted to the Kashmir soil The Forest Department could ultimately collect 6 lb. of seeds in 1938 from 2 oz. seeds obtained originally from Paris. After ensuring the successful germination of the seeds at Baramulla (5,500 ft.) the experiments were carried to suitable places at higher altitudes such as those ranging from 6,000 to 8,000 feet above the sea level.

The experimental cultivation continued till 1941 when a scheme of production on commercial scale was examined. The pyrethrum cultivation was thus put on sound economic footing under the direct control of the Forest Department. Suitable forest blanks wherever these were available, were converted into pyrethrum fields. The department had to overcome tremendous difficulties in planting pyrethrum on these blanks which were otherwise used as grazing pastures by the migratory tribes of Gujjars They vehemently opposed its cultivation because they considered it an interference with their grazing rights

Very little area of land belonging to private holders was used at first for pyrethrum cultivation. With constant propaganda however owners of private lands offered third or fourth rate land from point of view of food production for pyrethrum cultivation on rental basis in order to get some returns out of this poor quality land. Such land was fortunately found to be quite suitable for pyrethrum cultivation; in fact, the plants flourished in these areas even better than forest blanks.

Area under Pyrethrum.—The figures of acreage under this plant are available from the year when it was put on an industrial basis and the progress appears to be quite satisfactory especially considering that the propaganda of 'grow more food' was being pushed on during these years because of the prevailing war conditions.

TABLE I
Area under pyrethrum plant.

Year	*Approximate area in acres*
1941-42	322
1942-43	896
1943-44	1350
1944-45	1600
1945-46	2100

Cultural practice.—It may be said in a general way that the agricultural operations in connection with pyrethrum are similar to any other crop. The plant is perennial and if properly tended gives good quality of flowers for from five to seven years. In actual practice in Kashmir 6 to 7 seers of seeds per kanal (1/8 acre) are sown in well-prepared nursery beds during August-September or March-April. After four to five weeks the seedlings are transplanted on ridges to facilitate drainage

Transplanting is done in the spring or in the autumn. Care is taken in the autumn to transplant the seedlings firmly and deeply so that roots are not lifted up and exposed to frost that follows immediately after. Drizzling rain is observed to have very beneficial effect during transplantation periods and on the transplanted plants. Transplantation can also be done during summer if seedlings are frequently watered.

Weeding operations are tended in the spring and the autumn and are very advantageous to the growth of the plant. The plant flourishes on well-drained soil, in fact waterlogging kills the pyrethrum plants.

The plant begins to flower in June and continues flowering till the end of July. Flowers are harvested when the disc florets are three-fourths open These are generally hand-picked by numerous labourers and are dried in the sun on tarpaulins for three days after which they are removed into shade to complete the rest of drying.

Experimental work done at the Drug Research Laboratories.—Since the cultivation of pyrethrum was started on commercial scale, steps to put the industry on sound scientific lines were also taken. Series of experiments were conducted in the Drug Research Laboratories in collaboration with the Forest Department on different problems in connection with drying and storing of flowers. Some of them are briefly summarized below:—

(a) *Effect of altitude.*—Whereas pyrethrum can be cultivated at altitudes of 5,000 feet to 8,000 ft. above sea level, an altitude of 6,000 ft. represents the optimum height for the cultivation of this plant in Kashmir with a view to obtain the highest yields of pyrethrin. This is shown in the table given below.

TABLE I.

Effect of altitude on the pyrethrin contents.

Plantation ..	I	II	III	IV	V
Altitude ..	5000	5500	6000	7000	8000
Percentage of total pyrethrine ..	0.95	1.02	1 1	1.01	1.00

(b) *Method of drying.*—Series of experiments were conducted to investigate the best method of drying pyrethrum flowers under natural conditions. Samples of flowers were collected from plantations situated at different altitudes and were subjected to drying by the following four natural methods.

(A) Drying in sun.
(B) Drying in the shade.
(C) Drying in sun for three days and then completing the rest of drying in shade.
(D) Keeping in sun for seven days.

From, the perusal of the huge data collected during the course of this investigation it was observed that total pyrethrin contents were highest in the case of flowers dried for three days in the sun and then completing the rest of drying in shade.

The method of drying in shade was a lengthy one and could only be resorted to if weather conditions were bad. It appeared therefore, that the best method of drying pyrethrum flowers under the climatic conditions in Kashmir would be to dry them for three days in the sun and to complete the rest of drying in shade.

(c) *Effect of age of the plantation.*—An examination of the flowerheads in respect of the age of the plantation revealed a gradual fall in the active principle content from year to year. This is probably due to the exhaustion of soil and some other factors. The results are tabulated below.

Effect of age of the plant on the pyrethrin contents.

	1st Year.	*2nd Year.*	*3rd Year.*	*4th Year.*	*5th Year.*
Plantation I: Altitude 6,500 ft. ..	1.05	1.03	0.98	0.94	0.97
Plantation II: Altitude 8,000 ft. ..	1.05	1.04	1.02	1 01	0 98

(d) *Number of flowers per plant.*—In some of the pyrethrum plantations plots were laid out for counting the number of flowers on each plant The plants were divided into three categories, viz. heavy, medium and low, according to the yield of flowers. The maximum record of flowers in one plant was 714.

The heavy yielders if selected for seed only may ultimately prove of advantage in improving the yield of flowers per acre which is at present about 4 md (320 lbs.) against 8 md. (640 lbs.) recorded in Kenya, Japan and other places.

(e) *Development of flowers and harvesting time.*—It is an established fact that the flowers should be collected when the disc florets are three-fourth open. It has been shown by Chopra and co-workers that pyrethrum flowers growing in Kashmir conform to the general rule. Their results indicate that pyrethrin content tends to increase from the closed to the open stage But

when the flowers are fully open this percentage shows a decrease. This is obviously caused by the increase in weight of the flowerheads which follows pollination, and the subsequent formation of the seeds. The latter growth results in an increase of nearly 60 per cent in the weight of flowerheads

(f) Attempts were recently made to improve the quality of pyrethrum by introducing better seeds from Kenya, Japan and America. The seeds procured were sown and cultivated by the Drug Research Laboratory at its farms. The flowerheads when analysed for pyrethrin contents gave the following results:—

Source of seed.	*Total pyrethrum contents*
Kenya	1.25%
Japan	1.14%
U.S.A. . ..	1.03%
Locally acclimatised ..	1.07%

Animals and insects pests in Kashmir.—It has been observed that pyrethrum plantations on higher altitude—6,000 to 8,000 ft. in Kashmir are greatly damaged by rats. The rat which has been identified by the Zoological survey of India as *Microtus* (*Alticola*) roylei (Gray) resembles very closely *Microtus montosa* (True). The damage wrought by this rodent cannot be accurately calculated but it must be quite large Generally the two-year old plantation is attacked. The damage occurs during the autumn and winter when there is little other food for the rat and who then lives on the root of this plant readily available in the plantation. The rat burrows into the ridges on which the pyrethrum plants are transplanted to facilitate drainage This system of planting on ridges is very good from cultivation point of view but the rats find a good shelter in these and are able to inflict considerable damage to the plant.

So far local indigenous methods of controlling this pest have failed Experiments to control this rat with different chemicals and other biological controls are in progress

Recently an insect pest was also detected eating the root of some plants resulting ultimately in their drying up. The insect was collected in its larval stage and has been identified by the Forest Entomologist at Dehra Dun to be the larva of *Melolonthinie* of the family *Scaraboeideae*. It is one of the beetles whose habit is to feed upon or cut the roots of the plants. Fortunately it is not a widespread pest and measures are being taken to control it before it can do considerable damage to the crop

Marketing.—After the First World War, Japan became the principle exporter of pyrethrum in the world market and in 1935 maximum output was 12,500 tons. In 1933 Kenya began commercial production and is now the second largest producer with an output of nearly 2,000 tons in 1938. Brazil is the most recent newcomer to the pyrethrum export market and its produce in 1938 was 250 tons. The total world production of pyrethrum flowers is over 15,000 tons and America is the biggest consumer.

India has been importing pyrethrum flowers from Japan and Kenya till recently. During the Second World War the supplies from Japan were cut off and those from Kenya were restricted Kashmir then supplied pyrethrum flowers to the Government of India Although the quantities of flowers supplied were far too little for the total amount required for consumption, it did help to meet to some extent the military requirements during the war.

During 1941-42 when the Kashmir Government decided to expand the cultivation of pyrethrum on commercial lines under a special pyrethrum officer, the yield of flowers in the following year (1942-43) was about 336 md. as

against 28 md during the previous year (1941-42) Still more area was gradually added to the existing pyrethrum area and the yield of dry flowers in the year 1943-44 was 1,468 md The crop suffered from the inclement weather conditions in the following year and the output in 1944-45 was not more than, 1,436 md in spite of more land under the crop than before During 1945-46 however the crop yielded a better harvest and the yield had gone up to 2,200 md During 1947 when Kashmir was raided the whole economy was shattered and the Pyrethrum cultivation was given up. The Government of India have now taken the initiative to revive the cultivation of Pyrethrum for home consumption and work has also been started in Kashmir again.

Recently, when the Central Government Planning Committee met to consider the postwar requirements of India, pyrethrum was discussed and it was revealed that the annual requirements of pyrethrum flowers will be in the neighbourhood of 4,000 to 6,000 tons and were likely to increase to ten times this figure in the course of the next 15 years

Pyrethrum industry, therefore, appears to have promising future as the source of much needed vegetable insecticide in this country, both for destroying vectors of disease and plant pests. It will readily find its commercial application in agricultural and horticultural domains as a useful remedy against the insect and other pests.

It may be asked what is the need for vegetable insecticides when such powerful synthetic insecticides such as D D T gamexane and others are available The advantages of vegetable insecticides is that they are comparatively less toxic to both animal and vegetable life than synthetic chemicals substances Besides this some of them e.g. pyrethrines have powerful immediate knock down effects which are not met with in the synthetic insecticides Besides vegetable insecticides appear to be more suitable in combating the plant pests and parasitic diseases because they destroy these without being much injurious to the host plant.

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SECTION F

GENERAL.

THE STANDARDIZATION OF CRUDE DRUGS USED IN INDIGENOUS MEDICINE IN INDIA

by R. N. CHOPRA, *F.N.I.*, K. L. HANDA, I. C. CHOPRA *and* L. D. KAPOOR,
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INTRODUCTORY

It is well-known that the Indigenous systems of medicine in India still give medical relief to about 80 per cent of the population. In the majority of the rural areas this is the only type of relief available to the people even now. These systems also have a considerable hold on a large section of the urban population including the intelligentia as well. The materia medica of these systems is, therefore, largely used although it is well-known that the drugs in use are very often grossly adulterated. Hakims, Vaidyas and even some of the firms manufacturing preparations used in these systems, mainly depend for the supply of the crude drugs on ordinary drug dealers (*Pansaries*) in the Bazar. While giving evidence before the Committee on Indigenous systems of Medicine (1948) eminent practitioners of Indigenous medicine complained that they had great difficulty in obtaining genuine drugs. The great majority of those available in the market were invariably either heavily adulterated or entirely replaced by cheap and worthless substitutes dressed up to resemble the genuine article. All the witnesses and, particularly the leading manufacturing firms dealing with these medicines have expressed very great dis-satisfaction and have pressed for early action for the rectification of this state of affairs. It has been said that on account of difficulties involved in proper identification of medicinal plants, it is difficult to get genuine specimens of even commonly used drugs in the market. Many drugs are being sold under different names and different drugs under the same name and even the learned practitioners of Indian medicine cannot definitely say which is which.

The Indian materia medica at present has about 2,000 drugs including the household remedies used in different states. Of these, two hundred are of mineral origin, two hundred of animal origin and the rest are of vegetable origin. During Asoka's time the Hindu materia medica contained about 700 vegetable drugs which were used by the Vaidyas. They were all cultivated in gardens meant for the purpose all over the country and the time of collection, the parts used, methods of curing and preserving were prescribed. Since the number of drugs commonly used in those days was not large, most of them were cultivated and no elaborate descriptions appear to have been given for their identification. The physicians also identified and collected herbs for their own use themselves and compounded and dispensed medicinal preparations to their patients.

In course of time more and more drugs of vegetable origin were included in the indigenous materia medica and at the present time about 1,500 such vegetable drugs with alleged medicinal properties have been enlisted. Of these, roughly about 350 are commonly used in different parts of India. No precise descriptions regarding the identification even of this smaller number was recorded till recent years. There are of course some vague descriptions of plants but these are confusing and definite characters for identification are not available. Further, the science of pharmacognosy of the plants as known to Western medicine now, was unknown and definite identification was not pos-

sible. As a result of these vague and confusing descriptions some of the important drugs of Ayurveda are not traceable at all.

The drugs used in indigenous medicine are not only without any definite botanical descriptions, but there are also no chemical or biological standards laid down anywhere to evaluate the quality of the crude material or the finished product prepared from them as is done in the Pharmacopoeias of Western medicine. The only tests applied are colour, smell, hardness, etc. of the crude material.

Since there is no proper Pharmacopoeia in the modern sense in the Ayurvedic or Unani systems in which standards are laid to judge the quality of drugs and since there is no Government control over the sale of adulterated and spurious drugs, the drug dealers (*Punsaries*) take full advantage of this and sell to their customers anything that resembles or may be made to resemble the genuine drug. In fact the nefarious trade in adulterated and spurious drugs has reached such alarming proportions that it is often very difficult to get a sample of genuine drug in the market without special efforts. It would not be an exaggeration to say that there is at present hardly any drug for which a spurious substitute is not available in the market sold at, of course, comparatively much cheaper price.

In order to study the present position of the crude drugs used in the indigenous medicine as available in the market all over India, samples of some of the important drugs were procured with the help of learned Hakims, Vaidyas and manufacturing houses from different parts of India (Bombay, Surat, Madras, Travancore, Delhi, Amritsar, Srinagar, Jammu etc.). These were tested chemically, botanically and pharmacognostically and the results compared with the standards laid down in various publications. These studies revealed that most of the drugs sold in the market all over India were heavily adulterated.

None of the samples of extract berberis (*Rasaunt*), for instance, commonly used were of the required standard. Only 2 samples of extract of glycyrrhiza were genuine; the rest were either spurious or were adulterated Only one sample of catechu was of the required standard, all others were adulterated

The alkaloidal content of aconite roots varied from 1.03 to 4.7 per cent They were, in all cases, mixtures of different aconite species growing in India Similarly, a large part of *Tabashir* sold in the market was spurious having been prepared from ammonium and sodium silicates; only 2 among the samples examined were genuine. Many market samples of saffron, red lead, sandal wood and gum mastic were genuine but many also were spurious, in fact adulterated specimens were more common.

Pharmacognostic studies revealed that only 2 samples of *Banafsha* were genuine; the rest were either allied species of Viola, viz *V. sylvetica, V. canescence,* or other plants resembling Viola The herb sold as *Onosma bracteatum* is in reality any *Boraginaceae sp.* Out of the large number of samples of *Akarkara* roots examined, only 4 were found to be genuine. Roots of plants dyed with a brick-red dye were sold in the market as true *Ushba;* of the samples examined, only 2 were found to be genuine. Market samples of *quince* seeds, *Musli* and *Chireta* were mostly genuine. Work has been undertaken to formulate suitable standards for the more common indigenous drugs.

It is quite apparent from what has been said above that there is urgent need for establishment of some control on the sale of drugs used in indigenous medicine The difficulty so far has been that no standards have been laid down with which the drugs sold on the market can be compared. While it is quite obvious that it is not possible to lay down standards for the hords of vegetable and other products that are mentioned in the literature, it should be possible to fix standards for the commonly used drugs which do not exceed 200 to 250 at

the most. The Indian Pharmacopoeia List of 1946 laid down standards on lines laid down in British Pharmacopoeia for many drugs used in the Indigenous medicine. These were worked out by the Committee appointed by the Government of India with a view to their inclusion in Indian Pharmacopoeia under preparation. A research Institute for Indigenous Medicine has now been established in Jamnagar, which it is hoped is fully equipped and adequately staffed. It should be the duty of this Institute to prepare a list of the important drugs commonly used in Indigenous systems which are on the market and lay down standards on the same lines as has been done for the Indian Pharmacopoeia List. This work should not take long and should be taken up immediately.

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SUPPOSITORY BASES FROM KOKUM BUTTER

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(Communicated by B Mukerji, F.N.I.)

Suppositories, though an ancient form of medication, still maintain their place as very useful means for the administration of medicaments. This form of medicament may be intended for the treatment of localized diseases or for general absorption Medicines introduced into the rectum in a proper condition will produce their characteristic effects upon the system in the same way as when swallowed. Foods are also given in this manner in special cases, as after surgical operations or when the stomach is in a irritable or weak condition and is unfit for the reception of food or medicines.

The most commonly used base for the preparation of suppositories in temperate climates, is cacao butter. Though it has proved to be useful in several instances, it suffers from two great drawbacks. First, its transition temperature is close to its melting point and second, addition of phenol, salol or similar substances lowers its melting point to a great extent. Further, since cacao butter softens at 25°C and since the average room temperature in the tropics is about 30°C, suppositories made of cacao butter become too soft and often loose their shape in tropics.

Several attempts have been made to overcome these defects. So far as raw material cheaply avilable in India are concerned, it must be mentioned that kokum butter which is the fat extracted from the indigenous plant "*Garcinia indica*" shows characteristics resembling those of cacao butter. Gayatonde and Khorana mixed kokum butter with various other oils and fats and studied the products for the suitabilities as suppositories bases. Taking into consideration the melting points, transition points and consistency just below the melting point, the following three products were found to be the most suitable bases for suppositories—

(a) Kokum butter (35%) and cacao butter (65%)
(b) Kokum butter (55%) and ground nut oil (45%)
(c) Kokum butter (60%) and hydrogenated ground nut oil (40%)

For substances like phenol which lowered the melting point of the base kokum when used alone was found to be quite satisfactory. Modern trend in pharmacy is to have emulsion bases both for ointments as well as suppositories. Medicaments particularly antibacterials have been found to be more effective when mixed with emulsion bases than with the fatty bases. In the subsequent work therefore, emulsifying agents were added to pure kokum butter and the resulting mixtures tested for their usefulness as suppository bases. The emulsifying agents used were:—

(1) Triethanolamine stearate
(2) Lanette wax sx
(3) Wool alcohols.
(4) Carbowax 4000 and cetyl alcohol.

The percentages used were 5% and 10%. The melting points of the mixtures were always above 37°C, but this fact did not go against their usefulness, and they proved to be good bases when their behaviour under artificial body conditions was studied. These studies were carried out in a special apparatus wherein, the time for the disintegration of a suppository in contact with normal saline at 37°C and under constant pressure simulating the sphincter muscle was noted Results showed that bases prepared from kokum butter and emulsi-

fying agents could be classified in order of preference on basis of behaviour under artificial body conditions, consistency and keeping qualities, as follows.

(1) 10% Triethanolamine stearate.
(2) 10% Wool alcohols
(3) 10% Lanette wax
(4) 5% Triethanolamine stearate.
(5) 5% Lanette wax.
(6) 5% Carbowax 4000 and cetyl alcohol.
(7) 5% Wool alcohols.
(8) 5% Carbowax 4000 and cetyl alcohol

Micropenetration readings of these bases at various temperatures indicated that all of them remained quite firm upto 34°C but there were some differences in behaviour above that temperature Triethanolamine stearate base as well as the wool alcohols base softened rapidly between 36 and 37.5°C, while the softening of Lanette wax base started earlier and proceeded more gradually. In the presence of moisture all these bases emulsified rapidly at 37.5°C Under very slight pressure, as might be expected when the suppositories are inserted into the body cavities and in presence of normal saline at 37.5°C suppositories of the most of the bases were almost completely disintegrated and emulsified in 40 to 60 minutes Thus means that when inserted into the body cavities these suppositories would slowly disintegrate in about an hour's time and thereby supply the medicament to the body tissues gradually but continuously for at least an hour.

Further studies revealed that these bases could absorb considerable amount of water and still be quite suitable for making suppositories. The maximum amount of water that could thus be incorporated in each base is given below.

Kokum butter and triethanolamine stearate	(5%)	33%
„ „ „	(10%)	23%
„ and Lanette wax S.X.	(5%)	10%
„ and Lanette wax S.X.	(10%)	9%
„ and Wool alcohols	(5%)	30%
„ and „	(10%)	20%

With the maximum amount of water incorporated all these bases remained quite firm upto 34°C and softened sharply after that below the body temperature.

Thus a mixture of kokum butter (35%) and cacao butter (65%) is a much better suppository base than cacao butter alone In cases of phenol etc, which lower the melting point of the base kokum butter alone may be used When kokum butter is mixed with 5 to 10% of any of the emulsifying agents viz, triethanolamine stearate, Lanette wax S.X. and Wool alcohol, we get products which form satisfactory self-emulsifying suppository bases They can be used as such or with considerable amount of water incorporated, in some cases as much as 33%. Loss of water per week from these bases is quite low

Summary

This work deals with the use of Kokum butter as a suppository base. Three types of bases have been investigated namely mixtures of Kokum butter and Cacao butter, mixtures of Kokum butter and emulsifying agents and mixtures of Kokum butter, emulsifying agents and water. Results showed that Kokum butter can be used as a substitute of Cacao butter with several definite advantages.

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Group II.

INSECTICIDES.

VEGETABLE INSECTICIDES

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(Communicated by B. Mukerji, F.N.I.).

The annual loss caused to India through insect pests has been computed roughly at over a million and half of human lives and about 2,000 millions of rupees. One of the necessities for combating this menace of insect pests and to avoid this huge waste of national wealth is to find cheap and effective insecticides, for the diverse needs of agriculture, destruction of household pests, and prevention of vectors of such diseases as malaria and similar insect-borne diseases For many reasons vegetable insecticides are preferable to the mineral ones, as the use of vegetable insecticides should avoid the risks of phytotoxicity accumulation of harmful residues on plants and in soils and hazards of human and cattle health, and are undoubtedly less harmful. Most of the mineral insecticides used in India are imported from foreign countries and are therefore expensive and beyond the means of the great masses in India whose economic condition is very low. So far as the insecticides from vegetable kingdom are concerned, so little is known in this country, that we have to depend on these growing in other countries. The larger the number of effective insecticides we discover from among our plants, the greater will be the chances of their being brought into wide use by the people for medical, veterinary, household and agricultural purposes.

Among vegetable insecticides of proved value may be mentioned *Chrysanthemum* (pyrethrum), *Derris* (tuba root), *Nicotiana* (tobacco), *Tephrosia*, *Picrasma* (quassia), *Delphinium* (larkspur), *Veratrum* etc. In India, the cultivation of pyrethrum has been attempted recently. *Derris elliptica* is found wild to a very limited extent in India Roots from plants cultivated in Mysore have been found to contain a high percentage of rotenone. Several allied species found in India need investigation. Of these, *Derris ferrugnea* has been shown to contain rotenone and may prove to be a good insecticide Tobacco is largely cultivated in India. *Tephrosia vogelii* has been shown in foreign countries to be an efficient insecticide for flies, lice, and ticks, and it has been suggested that it may be used as a cheap commercial 'dip' for cattle. Some other species of *Tephrosia* are also stated to have insecticidal properties, but several of the Indian species although met with in abundance remain yet uninvestigated. Indian species of *Picrasma* also need investigation, and it is learnt that the powdered young leaves and twigs of *P. javanica* are used to kill mosquitoe larvae in Assam. Several Indian species of *Delphinium* are even now used for destroying maggots in wounds and may be [illegible] insecticides. Further-more, it has been stated that the alkaloid, cytisine is an important constituent of the Persian and Australian insect powder Thus alkaloid, which resembles nicotine in its action, has been found in at least six genera of which *Euchresta* and *Sophora* are represented in India

The investigation of insect-repellent plants is also of economic value and the cheaper and larger the number of such plants that could be used from amongst the indigenous plants, the more the chances of the people benefiting from their use. The roots of *Saussurea lappa*, the leaves of *Pogostemon*

heyneanus and *Azadirachta indica* are used to protect woollen fabrics from insects. The simple device of mixing of the leaves of *Vitex negundo* and of *Trigonella foenum-graecum* with the grains before storage, especially in rainy season, saves the grains from the ravages of insects Some essential oils such as citronella oil from *Cymbopogon nardus* and eucalyptus oil from *Eucalyptus globulus,* when rubbed on to the body, give relief from mosquitoe bites, for the period the odour lasts. Articles placed in boxes made from the wood of *Santalum album* are immune from the attack of insects. Investigation of suitable insect-repellent plants which, when grown, will keep away mosquitoes from the habitations has also engaged the attention of malariologists No really effective plant for this purpose has so far been discovered, but it may be worth while giving extended rials to absinthe (*Artemisia absinthium*), shrubby basil (*Ocimum gratissimum*) and other plants which diffuse strong fragrance in the surrounding atmosphere.

Investigation of vegetable insecticides and insect repellents from among the vast potential resources existing in India is likely to repay scrutiny. In India there are at least 75 plant species which grow wild in our hills and jungles or in a state of cultivation which are reported to possess insecticidal or insect-repellent properties. The brief information with regard to their uses and properties, their active principles so far as these area known and their distribution in India is as follows. In addition to these, a number of essential oil bearing plants may also be usefully investigated

1. *Acorus calamus* Linn. (Fam. Araceae) The aromatic rootstock is used to protect clothes from insect attacks. It is effectively used for the destruction of insects in powder form. The dry rhizomes contain 1.5-3.5% of an aromatic volatile oil. The roots contain also a glucoside named acorin. The plant is a semi-aquatic perennial herb found wild or cultivated throughout India, in marshy places and moist situations, ascending to 6,000 ft. in the Himalayas It is plentiful in the marshy tracts of Kashmir and Sirmoor, in Manipur and the Naga hills.

2. *Acorus gramineus* Soland (Fam. Araceae). The rootstock is employed in China as an insecticide and insectifuge. Contains an essential oil A semi-aquatic herb occasionally met with in Sikkim (up to 6,000 ft.) and the Khasia hills (4,000-5,000 ft)

3 *Adina cordifolia* Benth. & Hook. f. (Fam. Rubiaceae). The juice is used to kill maggots in sores. It contains a bitter principle The plant is a tall deciduous tree found in the sub-Himalayan tracts from Nepal eastwards to Burma and is common in the deciduous forests of south India, especially in the Eastern Ghats, Mysore and parts of Konkan.

4 *Agave americana* Linn. (Fam. Amaryllidaceae). It is said that the wall paper impregnated with juice of the leaves is proof against the ravages of white ants. The leaves contain an acrid volatile oil and the roots crystalline saponins. It is a shrubby plant indigenous to America and is planted in Indian parks and gardens.

5 *Anacardium occidentale* Linn. (Fam. Anacardiaceae). The juice is used to protect books, timber etc. from white ants. A black, caustic, oily juice [illegible] compound cardol, anacardic acid and an ether-soluble substance A tree introduced into India from Brazil, which has become naturalised in the West Coast.

6. *Anamirta cocculus* Wight & Arn. (Fam. Menispermaceae). A kind of ointment made from the drupes is used as an insecticide. Seeds contain

picrotoxin. The plant is a climbing shrub found in Orissa, Eastern Bengal, Khasia hills and Deccan.

7. *Annona reticulata* Linn (Fam Annonaceae). The leaves and seeds are considered insecticidal Alkaloid annonaine 0 03% found in the bark. The plant is a small deciduous tree of which some trees are found in the hills of south India.

8. *Annona squamosa* Linn (Fam. Annonaceae). The unripe fruit, seed, leaf and root are used for destroying insects and lice. Seeds contain an oil and a resin which contains an acrid principle. Leaves and seeds contain an amorphous alkaloid. Leaves, root and seeds contain hydrocyanic acid. The plant is a small tree which occurs wild and is also cultivated all over India.

9. *Arisaema speciosum* Mart. (Fam. Araceae) The tubers are used to kill worms which infest cattle. The juice is acrid. The plant is a herb found in the temperate Himalayas from Kumaon to Sikkim and Bhutan at 6,000-8,500 ft.

10 *Arisaema tortuosum* Schott (Fam. Araceae) The tubers are used to kill worms which infest cattle The juice is acrid The plant is a herb found in the temperate and sub-tropical Himalayas from Simla to Bhutan at about 8,000 ft., also in Khasi hills, Manipur, Bengal and West Peninsula.

11 *Aristolochia bracteata* Retz (Fam Aristolochiaceae). The juice is applied to foul and neglected ulcers to destroy insect larvae. Contains a nauseous volatile substance and an alkaloid. The plant is a slender herb, found in the upper Gangetic plain, Bengal, the western Peninsula, and in the north-west up to Bundelkhand. It grows abundantly on the black soil of the Deccan and Gujerat.

12. *Artemisia absinthium* Linn. (Fam Compositae). It is used to protect garments from moths. Contains volatile oil, a bitter glucoside absinthin and a bitter substance anabsinthin. The herb is found in Kashmir at 5,000-7,000 ft.

13. *Azadirachta indica* A. Juss. (Fam. Meliaceae). Dried leaves are commonly placed in books, paper, and clothes to protect them from moths etc The odour produced from the burning of the leaves is said to be fatal to the insects. Contains amorphous bitter principle and a crystalline substance, margosopicrin. Seeds also contain a bitter fixed oil. The tree grows wild in the dry forests of the Deccan and is cultivated all over India.

14. *Bambusa arundinacea* Wild. (Fam. Gramineae). The young shoots are lethal to mosquito larvae. Contain 0.3% of HCN. A tall thorny bamboo found wild throughout the greater part of the country, especially in the hill forests of western and southern India, ascending up to 3,000 ft. on the Nilgiris. It is also grown in other parts of India.

15. *Butea monosperma* Kuntze (Fam. Leguminosae). The powdered seeds are sprinkled over maggots to kill them. The seeds contain a small quantity of a resin and a large quantity of a water-soluble albuminoid. A medium-sized tree common throughout India up to 4,000 ft., except in very arid parts.

16. *Calonictyion muricatum* Dou (Fam. Convolvulaceae). The juice of the plant is used for destroying bedbugs. Seeds contain a resin. A large twiner found in the Himalayas up to an elevation of 5,000 ft., Gangetic plain, and Deccan hills.

17. *Cannabis sativa* Linn. (Fam. Cannabinaceae). The leaves or the whole plant are scttered under the bedsheet for driving away bugs. Contains a resinous substance which contain about 33 per cent of a toxic red oil. The

herb is practically naturalised in the sub-Himalayan tract in India, and is abundantly met with in waste lands from Punjab eastwards to Bengal and Bihar and extending southwards to Deccan.

18. *Cassytha filiformis* Linn. (Fam Lauraceae). It has been used as a wash in scald head and for the destruction of vermin. Contains the alkaloid laurotetanine. A parasitic leafless twiner, met with throughout the greater part of India.

19. *Centratherum anthelminticum* Kuntze (Fam. Compositae) The bruised seeds ground up into a paste with lime juice are used for destroying pediculi in the head and body in Travancore. The plant roasted in a room, or pounded and thrown about the floor, is believed to expel fleas A tall robust annual, distributed throughout India.

20. *Chrysanthemum cinerariaefolium* Vis. (Fam Compositae). The flower-heads are used in the form of powder or as a prepared extract for use as a household insecticides, as a livestock sprays and as horticultural dusts and sprays. Contain pyrethrin I and pyrethrin II. A perennial herb which has been successfully cultivated in India in Kashmir and in the Nilgiri hills, its cultivation has also given encouraging results in Kulu, Palampur, Mayurbhanj (Orissa), Kumaon, Assam, Mysore, Travancore and Kodaikanal.

21 *Chrysanthemum coccineum* Willd. (Fam. Compositae). This species is less active as an insecticide as compared with *C. cinerariaefolium*. A perennial herb grown to a limited extent in Assam.

22. *Cimicifuga foetida* Linn. (Fam Ranunculaceae). The roots are used to drive away bugs and fleas A tall perennial herb met with in the temperate Himalayas from Kumaon to Bhutan at an altitude of 7,000-12,000 ft

23 *Cinnamomum camphora* Nees & Eberm. (Fam. Lauraceae). Camphor is used to protect woollen clothes against insects and enters into the composition of several insecticidal preparations The plant is the source of camphor. A small tree introduced in India and planted in some gardens

24. *Citrullus colocynthis* Schrad. (Fam. Cucurbitaceae). The fruit is employed in Morocco for the purpose of protecting woollen clothing from moths. The pulp of the fruit contains an alkaloid, resins and a glycoside etc. Seeds contain about 15% of a fixed oil, traces of an alkaloid and an enzyme. The plant is a prostrate herb found throughout India in the warm, arid and sandy tracts of North-west, Central and South India and on the sea shores of the Coromandal coast, Gujerat, and other parts of Western India

25 *Croton oblongifolius* Roxb (Fam Euphorbiaceae) The oil from the seeds is sometimes used as an insecticide. A middle sized tree found in Sylhet, Chota Nagpur, Madhya Pradesh and West Peninsula.

26. *Croton tiglium* Linn. (Fam. Euphorbiaceae). The oil from the seeds is sometimes used as an insecticide. Seeds contain a most violent cathartic oil; also an alkaloid, ricinine, and two toxic proteins. A small evergreen tree distributed in Bengal, Assam, and south India either in a naturalised or cultivated state.

27 *Cucumis sativus* Linn. (Wild variety) (Fam. Cucurbitaceae). It has been stated that the juice banishes wood lice and fish insects. For this purpose freshly cut slices are strewn in their haunts. The fruits contain a proteolytic enzyme which resembles erepsin and also a bitter substance. A trailing or climbing annual widely cultivated throughout India. It is also found wild in north India.

28. *Curcuma longa* Roxb. (Fam. Zingiberaceae) Turmeric is used in

powder form to drive away ants by sprinkling the powder on the ant holes. Contains essential oil and alkaloid. A perennial herb cultivated in almost all the States in India, particularly in Madras, Bombay and Bengal for its rhizomes and constitute the turmeric of commerce.

29. *Cymbopogon nardus* Rendle (Fam. Gramineae). The leaves yield the citronella oil. This oil is not produced in any quantity in India, the requirements being met by imports chiefly from Ceylon. The oil is an important constituent of the various mosquito repellents sold in the market. A tall aromatic grass cultivated for the sake of its aromatic oil. According to some authors, this plant is also found in a state of nature in India.

30. *Cynanchum arnottianum* Wight (Fam. Asclepiadaceae). The leaves of the plant dried in shade and ground to powder are used for destroying maggots infesting wounds on animals. The plant is also said to be used as an insecticide. An erect plant found in Kashmir ascending to a height of 6,000-8,000 ft.

31. *Delphinium brunonianum* Royle (Fam. Ranunculaceae). The juice of the leaves is used to destroy ticks on animals, particularly for sheep. An erect simple herb found in the western Himalayas between 13,000-17,000 ft.

32. *Delphinium coeruleum* Jacq. (Fam Ranunculaceae). The root is used to kill maggots in the wounds of goats. An erect herb found in the alpine Himalayas from Kumaon to Sikkim and common in the Sutlej basin between 8,000-17,000 ft

33 *Delphinium elatum* Linn. (Fam Ranunculaceae) The seeds are used in Europe for insecticidal purposes Seeds contain alkaloids A small herb distributed in the western Himalayas from Kumaon to Kashmir at altitudes of 10,000-12,000 ft.

34. *Derris elliptica* Benth. (Fam. Leguminosae). The root known as derris or tuba root is an important article of commerce, being used as agricultural and horticultural insecticide. Derris owes its insecticidal properties to a group of compounds known as rotenoids, of which rotenone is the most important. Toxic substances other than rotenone, e.g. *dl*-toxicarol, tephrosin and deguelin also occur in the root. A large bushy climber; its cultivation on an experimental scale has been undertaken in Punjab (Gurdaspur), Madras (Coimbatore and Salem), Cochin, Travancore, Mysore and Assam.

35. *Derris ferruginea* Benth. (Fam. Leguminosae). The roots are insecticidal. Contain a fair percentage of rotenone. A woody climber distributed in the evergreen forests of eastern Himalayas and upper Assam.

36. *Duranta repens* Linn. (Fam. Verbenaceae). Macerated fruits yield a juice which even in dilutions of 1:100 parts of water is lethal to mosquito larvae; the action is less marked on culicine larvae. The juice can also be used as a larvicide in ponds and swamps. The fruits contain an alkaloid analogous to narcotine and the leaves saponin. An evergreen shrub widely cultivated in India as an ornamental hedge plant.

37. *Eucalyptus globulus* Labill. (Fam. Mrytaceae). The oil obtained from the leaves is largely used as a mosquito and vermin repellent and as an ingredient of insecticidal and insect repellent preparations. Contain essential oil. A large tree introduced into India; grows well in Nilgiris (5,000-8,000 ft.), Annamalai and Palni hills in South India, Simla hills (4,000-7,000 ft.) and at Shillong in Assam.

38 *Euphorbia antiquorum* Linn (Fam. Euphorbiaceae) The latex is used for killing maggots in wounds. The acrid latex contains caoutchouc A

fleshy shrub or small tree, distributed throughout the hotter parts of India ascending to 2,000 ft. in the hills. It is often grown for hedges.

39. *Euphorbia thymifolia* Linn. (Fam Euphorbiaceae). The oil is used as a spray to keep off flies and mosquitoes, and as a vermifuge for dogs and farm foxes. Yields a green essential oil A small prostrate annual met with throughout India in plains and low hills, ascending to 5,500 ft. in Kashmir.

40. *Gardenia campanulata* Roxb. (Fam. Rubiaceae) The fruit juice is an effective larvicide in dilutions up to 1:80. Contains saponin. A shrub found in the foot of the Sikkim Himalaya, Assam, Sylhet and Bihar

41. *Gaultheria fragrantissima* Wall. (Fam. Ericaceae). Essential oil from the leaves and other parts of the plant is an ingredient of various insecticidal and insect repellent preparations. A shrub met with from Nepal to Bhutan at altitudes of 6,000-8,000 ft.; also in Khasia Hills, Nilgiris, Pulneys and Travancore above 5,000 ft.

42. *Gloriosa superba* Linn (Fam. Liliaceae). In Guiana the juice of the leaves is used to destroy lice in the hair. Rootstock contains alkaloid colchicine, a toxic bitter principle, and two other bases A herbaceous tall climber met with throughout tropical India.

43. *Gynandropsis gynandra* Briquet (Fam. Capparidaceae). The seeds, rubbed with oil, are used to destroy head lice Contain an acrid volatile oil. An annual herb common as weed in the warmer parts of India.

44. *Hedera helix* Linn. (Fam Araliaceae). A decoction of the leaves is applied externally to destroy vermin in the heads of children. Almost all parts of the plant contain the glucoside α-hederin and probably other glucosides. Leaves also contain a saponin closely related to α-hederin. An evergreen climbing shrub found throughout the Himalayas at altitudes of 6,000-10,000 ft. and in Khasia Hills at 4,000-6,000 ft.

45. *Kalanchoe spathulata* DC (Fam. Crassulaceae). The leaves are said to be poisonous to insects. An erect perennial herb met with in the tropical Himalayas.

46. *Lagenandra toxicaria* Dalz. (Fam. Araceae). The plant is said to have insecticidal properties. Contains an acrid juice. An aquatic herb met with from the Konkan to North Kanara, Mysore, Coorg, Cochin and Travancore.

47. *Madhuca latifolia* Macbride (Fam. Sapotaceae). The oil cake is used as a worm killer for lawns—4 oz per sq. yard. During the burning of the oil cake the smoke which is produced is reported to kill insects. The seeds contain a neutral saponin. Leaves contain a glucosidic saponin and traces of an alkaloid. A deciduous tree found in Oudh, Madhya Pradesh, Madhya Bharat, Gujarat, Konkan, N. Kanara, S. M. Country and the Deccan, cultivated and self sown.

48. *Madhuca longifolia* Macbride (Fam. Sapotaceae). The residual cake, mowrah meal, after the extraction of the oil from the seeds, is employed as a worm killer for lawns. After extraction of the oil from the seeds, a sapoglucoside called mowrin is obtained from the residue. A large tree indigenous chiefly in the moist forests on the west side of India from the Konkan southwards to Travancore, Deccan, common in Mysore and cultivated in the Carnatic.

49. *Melaleuca leucadendron* Linn. (Fam. Myrtaceae). The essential oil obtained from the leaves and known as cajuput oil is a very good mosquito repellent and has the advantage over oil of citronella in that it volatilizes more slowly. A tall tree sometimes planted in Indian gardens

50. *Millettia auriculata* Baker (Fam. Leguminosae). The roots are applied to sores on cattle to kill vermin. A large woody climber found in the outer Himalaya from the Sutlej eastwards to Sikkim, up to 3,500 ft., Bengal and South to the Godavari.

51. *Nicandra physaloides* Gaertn (Fam. Solanaceae). In Madagascar, a decoction of the leaf is used to destroy *Pediculus capitis*. It is said to be used as a fly poison in some parts of the United States of America An erect herb native of Peru. It is often cultivated and is also found as a weed in many parts of India on rich soils up to 7,000 ft.

52. *Nicotiana rustica* Linn. (Fam. Solanaceae). Its uses and constituents are similar to the next plant *N. tabacum*. An erect herb cultivated in Bengal and some other parts of India.

53. *Nicotiana tabacum* Linn. (Fam Solanaceae). Tobacco is widely used in India against insect pests of the sucking type. In the form of dusts and sprays it is used against a variety of insect pests and is much used in horticulture. Tobacco seed oil has proved effective as an insecticide against some caterpillar pests in Hyderabad and some other States Leaves, stems and roots contain volatile alkaloid, nicotine Leaves contain also several other alkaloids and two glucosides An erect herb cultivated throughout India

54. *Nigella sativa* Linn. (Fam. Ranunculaceae). The seeds are scattered between the folds of woollen and linen fabrics to prevent them from being eaten by insects. An annual herb extensively cultivated in many parts of India, particularly in Bihar and the Punjab.

55. *Ocimum gratissimum* Linn. (Fam. Labiatae) The plant diffuses a strong fragrance in the surrounding atmosphere and is considered to be a good mosquito repellent Its plantation has been suggested as a measure of biological control of mosquitoes Contains essential oil, thymol, eugenol, and methyl chavicol. A shrubby perennial cultivated throughout India; said to be commonly wild in western India

56. *Pachygone ovata* Miers (Fam Menispermaceae). The dried fruit is uesd for destroying vermin. A lofty climber found in south India.

57. *Peganum harmala* Linn. (Fam. Rutaceae). The smoke from the burning plant or seeds is supposed to purify the air and is considered to be mosquito repellent. The powdered root mixed with mustard oil, is applied to the hair to destroy vermin. Seeds contain the alkaloids harmine, harmaline, harmalol and peganine and also a soft resin. A bushy perennial herb met with in the fields and waste places in the Punjab, Kashmir, Delhi, Uttar Pradesh, Bihar, Deccan, Konkan and Cutch.

58. *Picrasma javanica* Bl var. *nepalensis* (Fam Simarubaceae) The young leaves and twigs are used in Assam as a larvicide. A medium-sized tree found in Assam and Nepal.

59. *Pieris ovalifolia* D. Don (Fam Ericaceae). The young leaves and buds are used to kill insects. Contain a toxic substance. A deciduous shrub or small tree found in the temperate Himalaya, from Kashmir to Sikkim and Bhutan usually between 3,000-8,000 ft. and in Khasia mountains between times planted in gardens in Bengal and Bombay States.

60. *Pogostemon heyneanus* Benth. (Fam. Labiatae). The dried leaves are commonly used for scenting linen and other clothes and to ward off insects from shawls etc. Contains an essential oil An aromatic herb found in open forest land in Western Ghats from south Kanara southwards; often cultivated and then run wild Also in the Nilgiris about Kotagiri at 6,000 ft Sometimes planted in gardens in Bengal and Bombay States.

61. *Polygonum flaccidum* Meissn. (Fam Polygonaceae). In Assam the plant is used as a vermicide. The greenish mucilaginous juice of the plant kills off mosquito larvae in 15 minutes, but it is not lethal in dilutions. It is common throughout India in wet places, ascending the Himalayas to 4,000 ft

62. *Polygonum hydropiper* Linn. (Fam. Polygonaceae) It is said that the insects avoid this plant and that when dried and laid amongst clothes no moth will touch them The herb contains formic acid, acetic acid, baldrianic acid, much tannin and small amounts of an essential oil The root is said to contain oxymethyl-anthraquinones. A rather robust annual found in the plains as well as the hills in India.

63. *Randia dumetorum* Lam. (Fam Rubiaceae). In Konkan the bruised fruit is mixed with grain to save it from insect attacks. A 10 per cent aqueous extract of the root sprayed against the green scale of coffee gave an 80 per cent mortality of the insects in 4 days. The fruits contain saponin in the pericarp, a glucosidic saponin in the pulp, and the seeds are said to contain traces of an alkaloid. An essential oil is also present. A large shrub or small tree found throughout India

64 *Ricinus communis* Linn. (Fam Euphorbiaceae). The oil from the seeds known as castor oil is stated to be an active poison for flies. The seeds contain a fixed oil and a toxalbumin, ricin, which does not pass into the oil. A tall stout annual or perennial cultivated throughout India for its seeds particularly in Madras, Bombay and Bengal and is also naturalized in many parts of India.

65. *Ruta graveolens* Linn (Fam. Rutaceae). The plant is sometimes spread on beds to keep away the insects. The leaves, roots and seeds contain a volatile oil and also a glucoside, rutin, and a coumarin-like odoriferous principle. The plant is cultivated throughout India.

66. *Santalum album Linn.* (Fam. Santalaceae). The essential oil is an efficient insecticide and insect repellent. Small chips and raspings of the heartwood would serve the purpose of keeping off insects when placed among clothes. The heartwood of the tree contains an essential oil which is also present in the roots. A small evergreen tree met with in the dry, open regions of south India and in the Bombay State from Nasik southwards.

67. *Sarcostemma acidum* Voigt (Fam. Asclepiadaceae) The plant is often used by the farmers to extirpate white ants from sugarcane fields. A bundle of twigs is put into the trough of the well from which the field is watered, along with a bag of salt, hard packed, so that it may dissolve gradually. The water so impregnated has been stated to destroy the ants without harming the crop. It is a leafless, twining or trailing, jointed shrub usually found on arid rocks in Konkan, Deccan, Northern Circars, Carnatic and on Horsleykonda up to 4,500 ft. Also met with in Bengal and reported from Singhbhum, Puri and Ranchi.

68. *Saussurea lappa* C. B. Clarke (Fam. Compositae). The roots are used to protect woollen fabrics from insects. Contains an essential oil, alkaloid saussurine, resin and traces of a bitter substance. A robust tall perennial herb found in Kashmir and the surrounding country where it grows on moist slopes at altitudes of 8,000-13,000 ft.

69. *Schleichera oleosa* Merr. (Fam. Sapindaceae). The powdered seeds are applied to ulcers of animals for removing maggots. Seeds contain a fixed oil and small quantities of a cyanogenetic compound. A small or medium-sized tree found in Sub-Himalayan tract from the Sutlej to Nepal, Chota Nagpur, Madhya Bharat and south India.

70 *Scleria pergracilis* Kunth (Fam. Cyperaceae.) The lemon-scented leaves are used as mosquito repellents. The plant is widely scattered from Garhwal at an altitude of 5,000 ft. to Sylhet in Assam, Bihar, Chota Nagpur and the Deccan.

71 *Sophora mollis* R. Grah (Fam. Leguminosae). The seeds are stated to be useful for destroying vermin. It is likely that this plant contains the alkaloid sophorine which is identical with cytisine and which has been isolated from *S. tomentosa* Linn This alkaloid has insecticidal properties An erect shrub found in the plains and low hills of north-western India, from Hazara and the Salt Range to Kumaon and Nepal, up to 4,000 ft.

72. *Tephrosia vogelii* Hook. f (Fam. Leguminosae). The leaves are said to be an effective insecticide against lice, ticks and fleas, in the dry state they are employed as a flea powder. It has been suggested that the plant might be used as a commercial dip for cattle Leaves contain tephrosin and deguelin. Seeds contain tephrosin, deguelin, dehydro-deguelin, *allo*tephrosin, and *iso*deguelin The plant is reported to be cultivated in Assam by tea planters as a green manure

73. *Trigonella foenum-graecum* Linn. (Fam. Leguminosae). The dried plant is mixed with the grains stored up in bags, to save them from insect attacks during the rainy season. Contains the alkaloid trigonelline and essential oil. An annual herb found wild in Kashmir and Punjab and commonly cultivated in many parts of India

74. *Vitex negundo Linn.* (Fam. Verbenaceae). The leaves are laid over stored grain to keep off insects. Contain an alkaloid A large shrub found throughout India.

75. *Zanthoxylum hamiltonianum* Wall. (Fam. Rutaceae). A boiled fresh solution of the roots killed 100 anopheline larvae in 7 minutes It acts equally on anophelines and culicines but has no action on pupae. The diluted juice loses its potency after 3 days and becomes inert on the 5th day. An evergreen, scandent, armed shrub met with in Assam.

INDIGENOUS INSECTICIDES

by K. B. LAL, *F.N.I., Plant Production Adviser to the Government of India, New Delhi.*

Indigenous insecticides in India are mostly of vegetable origin. Of these, perhaps the most well known and internationally used is Pyrethrum, which has been grown in Kashmir, Kamaun hills and possibly in some other parts of India also. Tobacco, *Thevetia nerifolia, ak* (*Calotropis* spp.), *neem* (*Melia azadirachta*), castor, *dhatura* (*Datura alba*), *Nerium oleander*, *Lantana* spp., soap nut (*Sapindus mukorossi*), tomato and a host of other plants have been known to possess insec[illegible] Many years ago, the Forest Research Institute, Dehra Dun, [illegible] annotated and very useful list of Indian plants believed or kn[illegible] insects or fish. This document, which does not appear to have been published, should aid materially in planning future work on plants of insecticidal value.

Kerosine and diesel oils have been about the only indigenous materials of non-vegetable origin which have been used as insecticides. These may, however, be ignored in the present discussion, first, because for a variety of reasons it is very doubtful if their use on any extensive scale in plant protection work would be continued in future and, secondly, because their sole source of supply in India is the Assam oil wells which produce only about 5-6% of the total requirement of the whole of India. Some other indigenous materials, for example, rock phosphates, sand and lime, have been used as inert dusts for mixing with stored grains with a view to keeping the latter free from insect pests. These materials, however, are strictly speaking, not insecticides since they do not directly kill insects but prevent their development and multiplication through desiccation and mechanical action. Some what similar has been the role of coal, wood and cattle dung cake ashes in grain storage. Asafoetida has been a material of animal origin which has been believed to prevent insect pest attacks in storage godowns.

Of the insecticides of vegetable origin, tobacco leaves and stalks in the form of a water decoction have been extensively used in India as a contact poison against such sucking insects as aphids and psyllids. Though tobacco is sprayed generally in liquid form on plants, its precise action against insects has appeared to be in the nature of a fumigant. For this reason, tobacco sprays have been more effective at higher (80° F and over) than at lower temperatures. The great difficulty in its use has been to obtain tobacco leaves, stalks and dusts of known nicotine content on the basis of which required strengths of sprays may be prepared. Not only may different species and varieties of the genus *Nicotiana* have different percentages of the alkaloid nicotine in them but even the same plant may have different nicotine contents in its leaves, stem and roots. The stage of growth of the plant and soil and season may also affect the percentage of nicotine. To obviate these difficulties, a standardised product, nicotine sulphate (Black Leaf 40), has been on the market for a long time in India but its ready and dependable availability and price have not generally encouraged its use. An administrative difficulty in the case of tobacco has been that it is a commodity subject to excise duty on the assumption that it is used only for chewing and smoking. The duty, which makes a difference of about 100% in the price, is, however, waived if it is proved that the tobacco is needed only for insecticidal purposes. This may be possible in a government department for not for cultivators, since chewing and smoking of tobacco is a very common habit in village. Attempt has been made to solve this difficulty by mixing chillie powder with the tobacco which renders the latter unfit for consumption without materially affecting its insecticidal property

During the past thirty years or so, some research work has been conducted now and then in different parts of India on the use of plants or plant products for the control of insect pests In Uttar Pradesh a water decoction of the leaves of *Thevetia nerifolia* as spray proved effective against the nymphs and adults of the painted bug, *Bagrada picta* Fabr., and powdered leaves as dust against the grubs of the mustard saw fly, *Athalia proxima* Klug. Similarly, a water decoction of *Calotropis* leaves caused about 80% mortality of the young caterpillars of *Prodenia litura* Fabr., a pest of castor chiefly, and of the adults and nymphs of the mustard, aphis, *Siphocoryne indobrassicae* Das. Various preparations of *T. nerifolia,* however, proved ineffective against the last-named pest. In the Punjab, water or other extracts of oleander, *ak, dhatura, neem,* castor and other plants appeared effective against a variety of insect pests, mostly of the sucking type Various plants and vegetable oils have also been in use as insecticides in the States of Mysore, Hyderabad and Madras and pos-

sibly in other parts of India as well. In Hyderabad, tobacco seed oil has been found effective against the castor semi-looper

For insecticidal uses, vegetable oils can have superiority over raw plant products (flowers, leaves, stalks, roots, etc.) in that the former can be made available in standardised forms. The Hareourt Butler Technological Institute at Kanpur has attempted to standardise certain plant essences by describing their concentrations as 1-*mana*, 2-*mana*, 4-*mana* and so on. Thus 1-*mana* means that one pound of, say, rose water has been prepared by taking one unit weight of rose which is doubled in 2-*mana* preparations. Some similar system of grading plant preparations for insecticidal uses should be very helpful

The Report of the Five-Year Plan of the Government of India stresses the need for the increasing use of indigenous materials for controlling crop and other pests. There can be no two opinions about the desirability of such a practice Available information on plants and vegetable oils with insecticidal properties shows that their use in large-scale plant protection work may be possible and practicable. It is not easy to say, however, to what extent the indigenous materials would be able to compete with modern, chemical insecticides, specially synthetic insecticides of foreign manufacture. One of the serious objections, raised against the continued use of modern, synthetic insecticides, is the danger of their gradual accumulation or persistence in harmful forms and proportions in soils and on crops. Another danger is to the health of human beings and domestic animals feeding on plants or their products treated with highly poisonous, chemical insecticides, to which the Planning Commission have also drawn attention The use of insecticides of vegetable origin should avoid all these and other risks, e g., that of phytotoxicity

One of the greatest needs of the present-day insecticides is in regard to their compatibility with one another. Crop pests and diseases have increased so much in variety and numbers during recent years all over India that their control must entail a constant programme of dusting, spraying and other treatments practically all the year round. Under such circumstances, economy of operations is of the utmost importance so that the cultivator may effectively control more than one post and/or disease with the same operation on the same crop Indigenous insecticides can, therefore, have a better future than otherwise if either they are effective against more than one pest occurring at the same time on the same crop or two or more of them can be successfully combined to bring about the desired results. Some attempts to combine two chemical insecticides or fungicides to kill more than one pest or disease have already been made and given promise of field application.

The development of some modern, synthetic insecticides, chiefly of the chlorinated hydrocarbon and phosphorous groups, during the last decade has displaced many indigenous and other insecticides in pest control work not only in India but elsewhere also. Undoubtedly, synthetic insecticides are quick and effective in their results and, within limits, safe and economical to use, characteristics which account for their great present-day popularity. However, in view of the need, first, for insecticides to be easily and cheaply available for identical operations, indigenous materials for this purpose should receive adequate attention.

In order to consider indigenous materials for insecticidal uses, it is necessary to know the characteristics of an ideal insecticide. These may be enumerated as folows:—

(i) High toxicity to insects.
(ii) Quick toxic action (Knock-down effect).

(iii) Stability on treated surfaces (Residual effect).
(iv) Non-phytotoxicity.
(v) Selective action in killing insects.
(vi) Toxicity to as many stages of insects (eggs, larvae, adults) as possible.
(vii) Harmlessness to higher animals, including human beings.
(viii) Compatability with other pesticides.
(ix) Harmlessness to beneficial soil fauna and flora.
(x) Non-accumulation in soil (in the form used) if accumulation leads to ill effects on the plants grown in the soil.
(xi) Harmlessness to edible plant products.
(xii) Effectiveness in small bulks per unit of surface area or material.
(xiii) Reasonable stability under ordinary conditions of storage and transport.
(xiv) Freedom from obnoxious odours and from irritating action on human skin and system.
(xv) Non-inflamability.
(xvi) Non-corrosive action on metal and rubber parts of machines.
(xvii) Availability for use in as many forms as desired (dusts, wettable powders, emulsions) at economical price rates.

Obviously no single insecticide can satisfy all of the above requirements but in investigating materials for insecticidal purposes it is well to know the directions in which efforts should be made Many of the defects, e.g, those of phytotoxicity, accumulation of harmful residues in soils, toxicity to higher animals, etc., would be removed by the use of insecticides of vegetable origin. On the other hand, in matters of high toxicity to insects, use in small bulks, etc., chemical, specially synthetic, insecticides, may prove superior. In regard to selective action against different species of insects, compatability with other pesticides and stability under ordinary storage and transport conditions, materials of vegetable and non-vegetable origin may both prove promising

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CHEMICAL EXAMINATION OF PLANT INSECTICIDES: ROOT BARK OF *TEPHROSIA PURPUREA* PERS.

by S. RANGASWAMI *and* B. V. RAMA SASTRY, *from the Department of Pharmacy, Andhra University, Waltair.*

(Communicated by B. Mukerji, F.N.I.).

Tephrosia purpurea Pers. is a copiously branched perennial with firm ascending stems, small flowers, thin silky corolla and 5-6 seeded pods more than an inch long It can be easily distinguished from *Tephrosia lanceolata* Grah. by its colourless stems; the stems of *Tephrosia lanceolata* are coloured red when fresh. The extract of the whole plant is described as a diuretic and useful

in cough, asthma and tightness of the chest. The oil of the seeds is said to be a remedy for itches, scabies etc. An infusion of the seeds is said to be useful as an anthelmintic for children, while the root is considered a useful antidote to snake-bite (Nadkarni 1927). The plant was first examined by Clark and Banerjee (1910), who isolated rutin from the alcoholic extract of the leaves. In preliminary experiments we found that the alcoholic extract of the root bark was toxic to fish We have therefore examined the root bark in detail and our results are described in the present communication.

The plants required for the study were collected in 1951 round about Visakhapatnam where they grow along with some other *Tephrosia* species. For the identification of the species we are indebted to the Systematic Botanist, Agricultural Research Institute, Coimbatore.

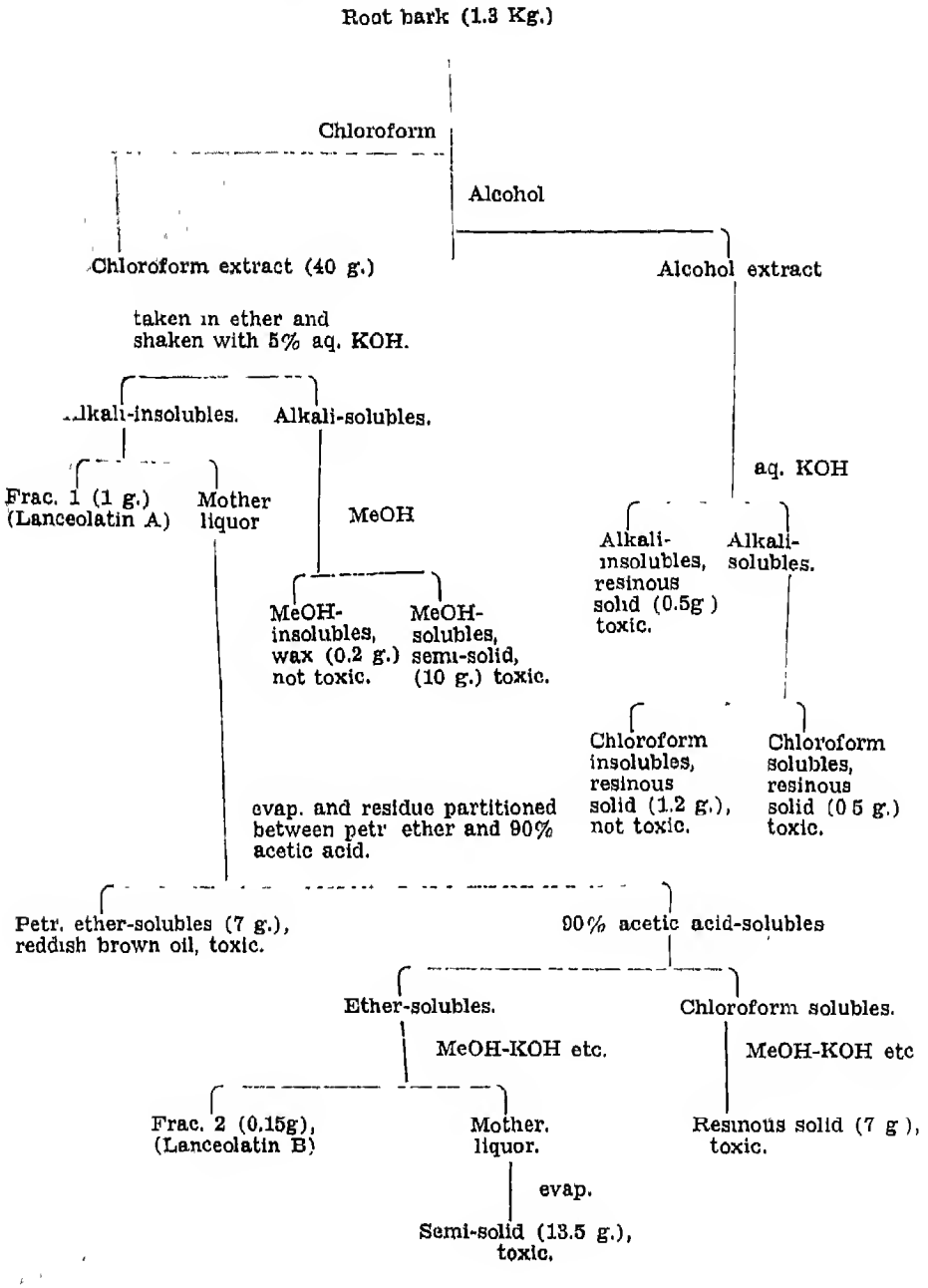
The powdered root bark was extracted successively with chloroform and methylated spirit. The total chloroform extract was divided into alkali-soluble and alkali-insoluble fractions by shaking an ether solution of it with aq. KOH From the alkali-insoluble fraction, a pale yellow crystalline solid was obtained It was identified as Lanceolatin A described by us under the name substance I in Part VI of this series (Rangaswamy and Sastry 1952). The amorphous residue recovered from the mother liquors was toxic to fish. It was divided into 90% acetic acid-solubles (neutral resin) and petroleum ether-solubles, according to the procedure adopted by Goodhue and Haller in their study of *Tephrosia virginiana* (Goodhue and Haller 1940) and by us in our study of *Tephrosia lanceolata* (Rangaswamy and Sastry 1952). The fraction soluble in 90% acetic acid could be further divided into two fractions, (1) ether-soluble and (2) ether-insoluble but chloroform-soluble.

The ether-soluble fraction, when boiled with methanolic KOH and worked up, gave a small yield of a crystalline solid; this was identified as Lanceolatin B, described by us in Part VI of this series under the name substance III The semi-solid residue obtained on evaporation of the solvent from the mother liquor of Lanceolatin B was toxic to fish

The ether-insoluble but chloroform-soluble part of the neutral resin did not yield any crystalline solid either on being taken into any of the usual solvents or on hydrolysis with methanolic KOH. The resinous semi-solid recovered after the hydrolysis was toxic to fish.

The alkali-soluble part of the total chloroform extract did not yield any crystalline compound. A small quantity of wax (m p 60-88°, non-toxic) separated on taking the residue into methanol. The semi-solid obtained on evaporation of the methanol was toxic to fish (30 p.p.m. in 20 minutes; 100 p p m. in 10 minutes).

No crystalline material could obtained from the alcoholic extract of the root bark. It was divided into three fractions (1) alkali-insoluble (2) alkali- and chloroform-soluble and (3) alkali-soluble but chloroform-insoluble. All of them were resinous and could not be crystallised from organic solvents No crystalline products could be obtained on acetylation or on degradation with 10% aq. KOH or on mild hydrolysis with 1% methanolic KOH. The first and the second fractions were toxic to fish (alkali-insoluble 40 p.p.m. in 23 minutes, 100 p.p.m. in 5 minutes; chloroform-soluble. 60 p.p m. in 60 minutes, 280 p p.m. in 14 minutes).



Experimental

Only the working up of the alkali-insoluble part of the chloroform extract is described here in detail.

Chloroform extract. The chloroform extract was taken up in 300 c.c. of ether and kept in the ice-chest. A small amount of resin sticking to the sides

was removed after two days by decantation. The clear ether solution was rapidly extracted with 5% aq. KOH (6 x 25 c.c.) and then washed with water, dil. HCl, and finally with water till neutral. Even during the washing with water a crystalline solid began to separate out, and the separation was complete within 24 hours. The solid (Fraction 1) was filtered, and washed with ether. The combined filtrate and washings were dried over anhydrous sodium sulphate, filtered and concentrated to 100 c.c. No further solid separated on standing or on dilution with benzene.

The solvent-free residue (28 g.), which was toxic to fish (8 p.p.m. in 24 min., 25 p.p.m. in 7 min) was treated with 150 c.c. of glacial acetic acid, 150 c.c. of petr. ether and 15 c.c. of water. The mixture was throughly shaken and the layers were allowed to separate. After drawing off the lower acetic acid layer the upper petr. ether layer was shaken with 3 x 30 c.c. of 99% acetic acid. All the acetic acid solutions passed in succession through a second, third and fourth separators containing 30 c.c. of petr. ether each. The combined petr. ether solution was washed neutral, dried over anhydrous sodium sulphate, filtered, concentrated to 50 c.c. and left in the ice-chest for a week. No solid product could be obtained. The remaining solvent was removed under vacuum. The oily residue (7 g.) was toxic to fish (50 p.p.m. in 48 minutes, 100 p.p.m. in 24 minutes).

The combined 90% acetic acid solution was diluted with water (1 litre) and the solution together with the precipitated resinous material was extracted with ether (3 x 100 c.c.). A part of the resin could not be extracted with ether. The aqueous solution together with this insoluble material was further extracted with chloroform (3 x 50 c.c.) (see next para). The combined ether solution was washed with dil. KOH, dil. HCl and water till neutral, dried over sodium sulphate, filtered and concentrated to 100 c.c., the remaining ether being removed under vacuum. The resulting ether-soluble neutral resin (14 g.) was taken in methyl alcohol (90 c.c.), treated with 10% aq. KOH (10 c.c.) and boiled under reflux for one hour, cooled and left in the ice-chest. Since no solid separated after 24 hours, the alkali was neutralised with 1:1 HCl, the solution diluted with water (200 c.c.) and extracted with ether (3 x 50 c.c.). The ether solution was washed free from acid, dried, concentrated to 50 c.c. and left in the ice-chest where upon a reddish brown solid separated. The mother liquor was decanted off, and the solid (Fraction 2) washed with ether by decantation. The combined mother liquor and washings were further concentrated and left in the ice-chest but no more solid separated. The reddish-brown semi-solid obtained on evaporation of the solvent under vacuum (13.5 g.) was toxic to fish (10 p.p.m. in 40 minutes; 50 p.p.m. in 10 minutes).

The combined chloroform solution described in the above para was washed free from acetic acid as described above and the solvent-free residue (7 g.) was refluxed with 1% methanolic KOH. Dilution with water, acidification, extraction with chloroform and evaporation of the solvent under vacuum yielded a residue (7 g.) which was toxic to fish (60 p.p.m. in 45 minutes, 100 p.p.m. in 27 minutes). No crystalline product could be obtained from it by taking it into any of the usual solvents.

Fraction 1 (*1 g.*) : On crystallisation from methylated spirit and then from methanol-acetone pale yellow needles, m.p. 187-89° were obtained. Colour reactions same as Lanecolatin A; mixed melting point undepressed.

Fraction 2 (*0.15 g.*). Repeated crystallisation from methyl alcohol yielded almost colourless needles which sintered markedly at 130° and melted at 145-47°. When the melt was allowed to cool, it solidified and on reheating the solid melted directly at 146-147°. When the crystals were dried in vacuum for one hour the melting point was 146-48° without previous sintering and there

was no depression in the melting point on admixture with a similarly dried sample of Lanceolatin B. The colour reactions of Fraction 2 and Lanceolatin B were identical.

SUMMARY

The isolation of two crystalline substance and a number of amorphous fractions, most of them toxic to fish, from the root bark of *Tephrosia purpurea* is described. The crystalline substances are identical with Lanceolatin A and Lanceolatin B which were first obtained from *Tephrosia lanceolata.*

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INDIGENOUS INSECTICIDES

by HEM SINGH PRUTHI, *F.N.I., Plant Protection Adviser to the Government of India, Ministry of Food and Agriculture, New Delhi.*

Estimates of losses to crops due to pests, diseases, rodents and wild animals and those caused by weevils and moulds to grains in storage in countries with a temperate and cold climates such as the U.S.A. are about 20 per cent of the total agricultural production. In warm countries, like India, where the conditions are more conducive for the rapid multiplication of such organisms, the losses are heavier. During epidemics of some pests and diseases, the losses are so colossal as to cause famines. Even at the rate of 20 per cent loss, the damage to Indian crops in aggregate works out at about 9 million tons per annum. Thus, it is certain that if we can prevent even half the losses which food crops suffer from the ravages of various enemies, the deficit of India's food supply can easily be wiped out Similarly we can substantially increase the production of our valuable commodities such as cotton and jute if their pests and diseases are kept under control.

Of the various method by which insect pests and plant diseases are kept under control in different parts of the world, the chemical method is the most common both in India and other advanced countries. The chemicals are used in the form of insecticides, fungicides, insect repellents, herbicides, seed dressings etc

The quantities of various chemicals used for crop protection purposes in some advanced countries e.g., U.S.A are immense. In India large scale operations for the control of pests and diseases were started about five years ago only. We have so far touched only the fringe of the problem. Even then, fairly large quantities of insecticides and fungicides have been used during the past year. Of these the most important are DDT (2,500 tons), BHC (about 2,000 tons) copper sulphate (21,000 tons), lime sulphur (200,000 gallons), Ethylene dichloride carbon tetrachloride (1,000 tons), etc. Almost all the insecticides

used in India are being imported at considerable cost in these days when there is shortage of foreign exchange. It is high time that we Entomolgists in India paid attention to this problem and with the assistance of chemists and chemical engineers produce large quantities of various pesticides at least for home consumption. This is all the more important since due to disturbed conditions all over the world, our supplies from foreign countries may be cut off at any moment. As a matter of fact, even during the past six months or so, the prices of important pesticides have increased by 25 to 50 per cent.

Of the various agricultural pests, locust is at present proving to be the most important enemy of Indian crops both those meant for food and those for other necessities of life, e g. cotton, jute etc. which are also high dollar earning commodities. Fortunately, we have in this country raw material for manufacturing several pesticides. Large quantities of rock phosphate, carbonates, coal tars etc. are found in different parts of India. It has been proved that pyrethrum which is a very important vegetable insecticide and which at the same time is harmless to man and his domestic stock, can be economically cultivated in several parts of India and in their insecticidal content the flowers compare very favourably with those grown in Japan, Kenya etc Again, nicotine sulphate of which the main basis is nicotine can be manufactured from tobacco leaves, stalks and other tobacco refuse etc which are available in large quantities in this country.

As regards synthetic insecticides like DDT, BHC etc, the main requirements are chlorine, benzene etc. which are often the by-products of other industries and therefore the manufacture of such insecticides in India should not be difficult. In recent years, several other synthetic insecticides have been evolved in other countries, viz., Toxaphene, Chlorodane, Aldrin, Dieldrin and so on. Synthetic hormone types of weedicides are also being produced in large quantities in U.S.A. and U.K As a matter of fact at present, bulk of world's pesticides are of synthetic origin and thus can be manufactured in any country

Copper sulphate, lime sulphur and some of the chemicals that are used alone or in combination as fungicides depend for their raw materials on the supply of copper, sulphur and mercury which are all imported at present But when mercury became scarced in U.S A. and the demand was large, the chemists of that country developed organic compounds of the carbonyl type, the principal among which was tetra-chloro-benzo-quinone type of fungicides known in trade as "Spergons" These types of fungicides (e.g. chloranil) have become very popular not only because of their low toxicity to the host plants but also of being highly toxic to the parasites Similarly it should be easy for the Indian chemists to develop such organic fungicides Since copper sulphate and mercury are getting scarce and their cost is increasing rapidly, every endeavour should be made by us to help the country by developing new compounds from materials which are readily available or from by-products of some industrial processes e.g manufacture of sugar, and thus make the country independent of foreign imports. As a result of the exten[illegible] thetic rubber industry in the manufacture of which large [illegible] had to be used, American chemists discovered that in [illegible] products there are organic sulphur compounds, derivatives of dithiocarbon acid, which have fungicidal properties. Such new fungicides e g 'Fermata', 'Zerlate', 'Dithane' etc. have come into great prominence in the U S A. and some of them are being often exported. India is importing large quantities for use in the control of some dreaded diseases like the late blight of potatoes etc. A large variety of 'seed dressings' have also been similarly developed

Insecticides are required for public health purposes as well as for the protection of our live-stock Most of the important human diseases viz malaria

cholera, typhoid, plague, typhus etc. are transmitted and spread by insects. Similarly is the case with regard to pests and diseases of live-stock. For the control of malaria alone thousand tons of insecticides are required.

The problem of evolving new and better insecticides, fungicides weedicides etc. should therefore form the main work of a Central Insecticides Laboratory This Laboratory should also test and standardise all the insecticides which are being sold in the market. At present the insecticides of these firms which can advertise the most are considered the best and there is no arrangement for testing the products to confirm the claims made by the importing firms. Furthermore a survey should be made of raw materials which can be at once used for manufacturing insecticides and those which with slight alteration in their chemical composition can be made to serve as insecticides or fungicides. This will make the country self-sufficient in these valuable products which are essential for increasing agricultural production and for the economic development of the country.

USE OF PYRETHRUM IN INSECTICIDAL AND RELATED FIELDS

by B. MUKERJI, *F.N.I. and* T. D MUKERJEA, *Central Drug Research Institute, Lucknow.*

The arrival of D.D.T and other 'residual' insecticides of high toxicity and more lasting in their potency, introduced a competitor to pyrethrum, the most popular insecticide known for some time past. This has given rise to a feeling in the minds of many that insecticides from plant sources would have increasingly less and less use and that there is hardly any need now of thinking about the possibility of cultivation of pyrethrum in India. The demands for insecticides have also decreased considerably with the termination of the 2nd World War, and in view of the tremendous output of synthetic insecticides, there is probably no future whatsoever for pyrethrum as a crop of commercial importance. This review is intended to throw light on the very extensive fields of utility of pyrethrum and its varied applications. It is hoped that this will create interest in the minds of several pyrethrum growers in India so that they can feel reassured as to the future commercial possibilities of this important insecticidal plant.

1. *Use in Household pests*: The first commercial outlet for pyrethrum was in the form of finely ground flowers marketed as a proprietary article for use as an insecticide against household pests and vermin. The insecticidal effect of pyrethrins are exceedingly rapid and they have been very widely used on account of their remarkable "Knock down" effect which is due to the rapid paralysis induced in contaminated insects. Pyrethrum powder alone, or reinforced with other insecticides or with activating synergists is still widely used for this purpose. The chief pests concerned are human parasites, cockroaches, clothes moths and carpet beetles against which the dry powder can be effectively used

2. *Use in the eradication of House Flies*: The ready destruction of the more elusive house flies of several kinds, mosquitoes, gnats and other flying pests of domestic and farm premises had to wait until it was recognised that the toxic constituents of the powder, the 'pyrethrins' when dissolved in deodo-

rised kerosene, could be used as an effective hand or power spray, with strikingly rapid action against these menaces to health

Synthetic organic insecticides e.g. D.D T. is highly toxic to houseflies but requires at least 10 min. to knock down, where as pyrethrum in combination with D.D.T. becomes a more potent insecticide by its fast knock down property.

3. *Cockroaches*· Domestic infestations of these pests are trivial compared with the plagues (often of more than one species) which invade bakeries, commercial kitchens, warehouses, sewers and ships, and are a danger to health Pyrethrin sprays have an immediate effect on cockroaches that the chlorinated hydrocarbons (synthetic insecticides) lack, flushing them from their hiding places and rapidly knocking them down (McGovarn 1946). In all circumstances involving risk to humans or animals, and to food, pyrethrum powder or spray, synergised or not, is a safe control. Because to warm blooded animals and human beings the pyrethrins are comparatively innocuous which makes them valuable for domestic use. In suitable cases, pyrethrum containing a small proportion of D.D.T. or B.H.C. is effective, the pyrethrum causing the cockroaches to emerge from their hiding places to meet the full effect of the spray.

4. *Other types of Flies*: Cattle sheds, dairies and stables are notoriously infested with blood-sucking flies, e.g, stable flies, horn flies and horseflies, as well as with houseflies, the pests if uncontrolled affect the health of the animals and in the case of dairy cows milk yield may suffer, while beef cattle become emaciated and "poorly". Where direct spraying of livestock is concerned, and the hazard of body fat and milk contamination is indicated pyrethrum formulations with synergists are favoured. Farm buildings, therefore, offer another field for pyrethrum sprays which are now commonly reinforced with piperonyl butoxide and applied as mists against the insects on the wing, and for spraying the surfaces of the premises and, above all, the breeding places of the insects.

5 *Mosquitoes* (*Anopheles, Aedes*) The following tribute to pyrethrum is quoted from Dr Muirhead-Thompson's recent book *Mosquito behaviour in relation to Malaria transmission in the Tropics* (1951) p. 88 "... . it is often difficult to realise what an important part was played till very recently by Pyrethrum in the development of this method of attack At the moment, apart from the routine treatment of aircraft, the use of Pyrethrum has been almost entirely abandoned, and yet for about ten years—roughly from 1935 to 1945—it was a method of control used in practically all parts of the tropics—sometimes the only method used Whether its use will ever be revived it is difficult to say, but in the course of that ten years it gave rise to a great deal of critical research, and stimulated lines of enquiry which have been of the greatest value in interpreting present-day work on the residual insecticides." A report by Drs. van Riel and Hoffmann (1948) records remarkable success against malaria mosquitoes in the Belgian Congo where a fine pyrethrum powder containing 1.3 per cent pyrethrins, was sprayed by powder guns on to the walls and ceilings of huts twice weekly for 1½-3 months. The treatment "assured the complete supression of anopheles in these huts."

The powder was also shown to have repellent action. "Vanishing" creams containing pyrethrin extract were used in the War to repel mosquitoes but were discontinued when more effective chemical repellents were discovered In this connection it is stated that effective personal protection against mosquitoes for seven hours can be obtained if pyrethrins are compounded with tragacanth gum as a base instead of with the usual paraffins.

6. *Disinfestation of Aircraft*· Aircraft are certain to be invaded by insects indigenous in the countries in which the planes touch down. If these countries contain insects known to be carriers of dangerous human or animal

diseases, e.g., the mosquito vectors of malaria (*Anopheles* spp.) and yellow fever (*Aedes aegypti*), it is vitally important that the aircraft should be completely rid of all insects before leaving for other countries, partly in the interest of the passengers, but chiefly to prevent the introduction of infected carriers into countries free from them. A second treatment on or before arrival is a further safeguard. Stringent world regulations now require a disinfestation by aerosol sprays of approved insecticides of all aircraft at key points of their flights. Such insecticides must be, *inter alia*, highly efficient and quick-acting, harmless to passengers after spraying, and non-inflammable. The case for the use of pyrethrum is obviously strong and this insecticide, reinforced with a synergist and with D.D.T. is an essential constituent of the standard formulations now employed (Busvine, 1952).

7. *Stored Products*: Insecticides may well prove to be an important factor in supplementing the available supplies of human and animal foodstuffs, notably cereals, since a large part of the world's crops is destroyed by insect pests either in the field or in the warehouses and granaries in which the products are stored The low risk to man and animals attending the use of pyrethrum renders it of unique value in the protection of stored products The insecticide may be employed as a dry powder, either alone or mixed with an inert dust, or as an oil spray. The latter method has been much used in several tropical and semi-tropical countries mainly for cleansing warehouses already infested. Warehouse sprays have a higher pyrethrin-content than household fly sprays since, in addition to relatively susceptible pests such as grain moths (e g, *Ephetia* and *Plodia*), they have to cope with more resistant beetles. At present these sprays usually contain 1.3 per cent pyrethrins when dealing with beetles and 0.65 per cent when required only against moths.

8. *Grain*: The use of finely ground pyrethrum powder applied directly to wheat and other grain has been the subject of valuable experiment in recent years. Beckley (1948) and Gnadinger (1936-45) showed that pyrethrum powder, either alone or mixed with an equal part of diatomite, added to clean wheat at a rate of 1-lb. pyrethrum to 200 lb. wheat in bags, kept the grain free from *Calandra* and *Tribolium* beetles for 8½ months, and destroyed almost completely insects infesting weevily wheat Further experiment indicated that, with ordinary commercial fine powder, 8 oz. per 200 lb. wheat would give adequate protection, while with very fine powder 2 oz would suffice.

9. *Groundnuts* are subject to destructive insect attack. The chief pests are the beetles *Tribolium* and *Trogoderma* and the larvae of the moths *Ephestia* and *Plodia*, all of which infest mainly the outer bags in the pyramids Trials have been made of spraying the stacked bags under tarpaulins with fine mists of gammexane-in-oil with the addition of concentrated pyrethrins to cause the beetles to leave their hiding places. More definite results are reported from mixing ½ lb of pyrethrum powder with the contents of a 189 lb bag, when an average saving of about 1.5 per cent of the weight of the nuts was obtained.

10. *Tobacco leaf* and manufactured tobacco, in store, frequently suffer much damage from the tobacco moth (*Ephestia*) and the cigarette beetle (*Lasioderma*). In the United States pyrethrum powder has been found fairly useful against the moth but the beetle is resistant.

11. *Horticultural Uses*: Pyrethrum has long been used as a horticultural insecticide, and, being harmless to animals, is specially valuable for treating foodcrop plants. Broadly speaking pyrethrum is used in two classes of application, viz., on field and on smaller specialized cultivations. An example of the former is in the control of coffee pests—the capsid bug *Lygus* and the variegated coffee bug *Antestia*—in Kenya (Gnadinger, (5).) The original

treatment of these pests with a pyrethrum-kerosene spray has been superseded by mechanical dusting with pyrethrum powder either alone or diluted with an inert carrier. In the Congo, pyrethrum powder mixed with wood ash has been successfully employed against the coffee bug (*Antestia*). The second case is illustrated by the use of pyrethrum in England for combating glasshouse insects. A powder consisting of pyrethrum and an inert carrier is regarded as probably the best antidote to the "greenfly" (aphis) so troublesome in commercial glasshouses; it has been very effective against the aphis attacking winter lettuce.

Toxic hazard to Man: Like D.D T. and most of the other organic insecticides pyrethrum exhibit no chronic toxicity. The oral toxicity of pyrethrins is so low that they have been used as anthelminthics (Chevalier 1930). Pyrethrins offer no residual hazard, particularly since their deposits are labile. They may, however, present certain unexpected hazards to the user, particularly to those who are subject to the "pyrethrum idiosyncrasy". Pyrethrum dusts produce allergic attacks in many persons who are sensitive to ragweed pollen (Feinberg 1934). Cases which resemble anaphylactic shock and cases of dermatitis have been recorded in the pyrethrum industry (Cox. 1944).

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